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Ban the bomb—properly

At long last there is a sign of life in the test ban business. For so long a sleeping partner among arms control proposals, some form of treaty to control underground nuclear weapons tests now looks like becoming a reality.

Underground weapons tests do little or no damage to people or property, but they do cause some offence on the international scene, particularly to countries such as India and Sweden who are exercising self-restraint in not going nuclear themselves. It must be surmised that the Soviet Union can see a diplomatic gain in negotiating now. Certainly Mr Nixon must see the advantage of being seen again as an international peace maker.

There are many issues which need discussion before the wheels of diplomacy turn too far. One such is the treaty that will be negotiated. There are three types:

- Thou shalt not (comprehensive).
- Thou shalt not be found out (technical).
- Thou shalt keep it in moderation (threshold).

Politicians would say that these three types represent increasing realism on the scientific aspects of monitoring. They also represent increasing cynicism and expedience of the negotiating parties.

There are many possible weapons so small that their testing could proceed unchecked except by espionage. A comprehensive treaty would rule such tests out; violation of the treaty would be a dangerous pursuit if only because the whole machinery of espionage could be brought to bear, with some justification, on exposing infringements. It may thus be politically unacceptable, not only as signing away nuclear rights but also as a treaty too easily abrogated.

At the other end of the spectrum, a threshold treaty would lay down a seismic magnitude above which a test may not be. This is a catastrophic procedure. For a start it ties matters of international security to a hopelessly complex scientific art. In 1968, the occasion of the last east-west scientific discussions on this matter, Russian and American delegates eventually had to agree that they always came up with a different number for the same event and an arbitrary correction had to be applied to prevent the discussions from breaking down altogether. When scientists sit down to write the language of any future threshold treaty they may again be seriously at odds—perhaps to the jeopardy of the treaty. Further, a threshold is a challenge to any nuclear country to improve its ability to decouple explosions from the geological surroundings, by firing in loosely compacted rocks or ultimately in large holes. With technological ingenuity explosions as large as 50 kilotons could be muffled to have a seismic magnitude of only 4.75, a widely discussed threshold value. Thus a threshold treaty would be gradually eroded by a determined nuclear power, and in time could become almost meaning-

less. And if the purpose of a treaty is to convince potential proliferating countries that the super-powers have a highly moral approach to nuclear weaponry, leaving open the door to tests of tens of kilotons will impress nobody. Finally, a threshold treaty will put back even further prospects for a comprehensive treaty.

If a comprehensive treaty is politically difficult, and a threshold treaty is meaningless, it is desirable to consider seriously a technical treaty. The onus is on a nuclear country not to be caught in activities it regards as vital. There is a continuing pressure on monitoring systems to improve, so the treaty is not so easily eroded. The treaty is not as vulnerable to violations as a comprehensive one would be, for the occasional detected test would be the subject of a discreet hint, not a diplomatic incident. One can hardly claim that a technical treaty is ideal—it too encourages an evasive approach by nuclear countries and it is not entirely inoffensive to non-nuclear countries but it represents a realistic goal for negotiation in the immediate future.

Negotiation on a treaty must not be impaled on the inspection issue. The concept of an annual ration of inspections must surely go; if inspections are needed they must be rare and subject to flexible arrangements.

Finally, explosions for peaceful purposes. In the United States these have made little headway, but the Soviet Union has a vigorous programme involving many explosions every year. Those within the United States who do not wish any treaty—and they include much of the atomic energy establishment—will point out that peaceful shots can be the guise for military tests (at least in the Soviet Union). If peaceful tests are seen as latter-day Trojan horses, then it is better that national programmes be stopped and resumed later under international auspices.

100 years ago



Col. Strange says :—

"I think it is an absolute necessity on the ground of my second postulate, in which I say that all science should be cultivated, even branches of Science which do not appear to promise immediate advantage. It is one of the most important parts of Science, and cannot be omitted without detriment to all the other parts. . . . Investigations connected with almost the whole of our material economy are required. There is no question connected with sanitary improvement, with water supply, or sewage, or telegraphy, or the enormous number of the requirements of the army and navy, which would not derive advantage more or less from investigations of a physical nature such as would be conducted in a physical laboratory. I think that the whole of our naval and military and social economy is dependent upon investigations such as would be carried on in a physical laboratory."

From *Nature*, 10, 21, May 14, 1874.



Future of forestry in Britain

A government decision about the future of British forestry is expected shortly. John Gribbin reviews the possible development of forestry in Britain in the light of the recently published annual report of the Forestry Commission.

THE future organisation of forestry activities in Britain has been under consideration now for several years.

The Committee of Public Accounts of Session 1967-68 expressed concern over the substantial losses of the Forestry Commission and the poor prospects for the future. It was at that time that there was a call for a proper consideration of whether the commission's noncommercial activities produced sufficient social benefits to justify the heavy expenditure. So the feeling at government level of a need for a complete overhaul of the Forestry Commission can certainly be traced back six years, and the overall review of forestry in general stems from this careful look at the Forestry Commission.

A White Paper published in June 1972 outlined the direction in which the government saw forestry developing, and recognised the need for landscape improvement and maintaining the character of traditional hardwood areas. But in itself that review had little practical effect, although at about the same time smallwood planting grants were suspended and replaced by an interim grant scheme to bridge the gap until a new structure had been worked out.

By October 1973 Mr Anthony Stodart, the then Minister of State at the Ministry of Agriculture, Fisheries and Food (MAFF), was able to report to the Commons that a general framework for the future of forestry policy had been worked out, and details were then expected to be announced early in 1974. But the General Election and resulting change of government has delayed announcement of a detailed policy; in the House of Commons on April 11 Mr Stodart asked Mr Roland Boyle, the Secretary of State at MAFF who now has responsibility for forestry, whether a decision had now been reached, and was told that "consultations are still in progress". Pressed by a Member concerned that woodland owners had no idea what to do about the 1974-75 planting programme

because the decision had not been taken, Mr Boyle replied "we have the matter very much in mind and hope to be able to announce a decision as soon as possible". Since then, the Forestry Commission *Annual Report and Accounts 1972-73* has been published (HMSO, 90p); together with previous government statements this makes it possible to foresee the likely development of forestry in Britain once the awaited government decision is announced.

One key change which has already been made is a reconstruction of the commission's accounts. From 1919 to 1972 the accounts have had to bear interest charges at the appropriate government lending rates, and these have averaged about 5% compounded over that period. Under the new structure the aim is to be a rate of return on funds of 3%, which is accepted as the maximum that forestry can be expected to earn in northern Europe on the best sites close to markets. That is an important proviso, for much of the commission's planting has been undertaken for strategic or social reasons, and cannot be expected to provide such

a high return. "Where less profitable activities are undertaken for reasons of government policy", says the report, "the commission will be given . . . subsidies each year to bridge the gap between 3% and the expected rate of return".

This policy is not affected by Britain's entry into the European Economic Community (EEC), which has no common forestry policy. Regulations to implement two EEC directives concerning improvement of genetic and other standards of seeds, young plants and so on became effective on July 1, 1973, and the EEC Commission is considering a draft directive which, although chiefly devoted to improving the structure of agricultural holdings, has some implications for forestry. But otherwise the British government has essentially a free hand in forestry matters.

It must be remembered, of course, that only half of the total productive forest area in Britain is owned by the commission; but in practice the interests of private and state forestry are complementary and cooperative, rather than competitive. A major difference

Table 1 Land use by Forestry Commission at March 31, 1973 (Thousands of hectares*)

	Great Britain	England	Scotland	Wales
Total area	1,212.0	306.7	744.6	160.7
Forest Land: Total	882.1	261.7	481.9	138.5
Under plantations	768.3	239.5	399.5	129.3
To be planted	113.8	22.2	82.4	9.2
Other Land: Total	329.9	45.0	262.7	22.2
Nurseries	0.3	0.1	0.2	—
Agricultural and grazing	151.5	14.2	123.2	14.1
Forest Workers Holdings	5.1	1.1	2.9	1.1
Unplantable and miscellaneous	173.0	29.6	136.4	7.0

* 1 hectare = 2.471 acres

Table 2 Planting in the year ended March 31, 1973 (hectares)

	Total	New planting	Restocking
Great Britain	23,158	19,378	3,780
Conifer	22,865	19,224	3,641
Broadleaved	293	154	139
England	3,154	1,735	1,419
Conifer	2,890	1,608	1,282
Broadleaved	264	127	137
Scotland	17,764	16,174	1,590
Conifer	17,739	16,151	1,588
Broadleaved	25	23	2
Wales	2,240	1,469	771
Conifer	2,236	1,465	771
Broadleaved	4	4	—

Conifers continue to dominate planting by the Forestry Commission, but that pattern is likely to be changed when details of the new government policy are announced.

Peeled woodwool billets stacked for air-seasoning in a clearing of 47-year-old Scots Pine at Bramshill Forest, Camberley.

is that private forests are concentrated on 'traditional' areas whereas the commission has afforested wide areas formerly in marginal agricultural use. The Forestry Committee of Great Britain, which represents private forestry in discussions with the government, generally sees eye to eye with the Forestry Commission, although it is in a better position to speak out against government proposals even to the extent of commissioning its own investigation of the accuracy of government studies relating to forestry. Of course, the two sectors of the forestry industry are in a competitive situation in the 'market place', but that market is so hungry for timber that both sectors are assured of selling their product.

Where the private sector differs from the Forestry Commission, however, is that small planters are in a much more vulnerable position when there is an air of uncertainty about the future direction the industry is expected to take. This is highlighted by the fact that the interim scheme for smallwood planting grants has now run its course, and that since the beginning of this year there has been nothing to replace it. A spokesman for the Forestry Committee said last week that small owners are already being affected, and that "some nurseries are finding it very tough" as a result.

This is a matter of more immediate urgency than the question of what should be planted in the 1974-75 season. For the private sector, the 1973-74 planting season is essentially over, and although there is a clear need for plans for the next season to be put in hand as soon as possible, there is probably less urgency about this at present than at any other time of the year. Of course, there can be little doubt that the detailed government plans will include a hardwood planting supplement—but it is up to the individual owner or planter to decide whether this is attractive enough for him to change his pattern of planting. All this takes time, and the present situation of uncertainty is clearly undesirable.

But the most important fact which must be taken into account when the government announces its new detailed plans is that in forestry terms Britain is very nearly saturated, with about 8% of the country covered by forest. Most of the figures in Table 1 are slightly down on last year; on March



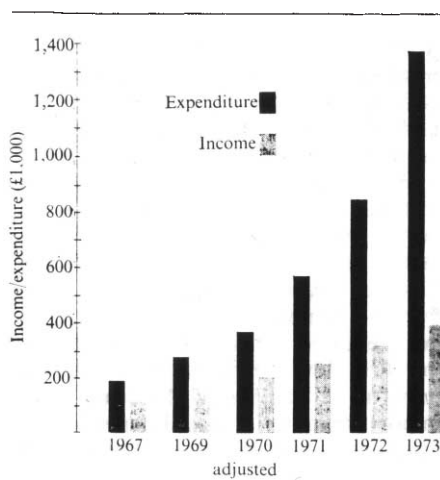
31, 1972, for example the corresponding figures for forest land and total area were 876.2 and 1,215.0 thousand hectares, respectively. The total area of forest land acquired in the year ended March 31, 1973 was 6,000 hectares—more than 10,000 hectares less than in the previous year, and some 15,000 hectares less than in 1971-72. One reason for this is improvements in the sheep farming industry which make less land available for forestry; by and large, there is no longer any suitable land on the market.

In the light of this, and the removal of forestry's strategic role, the future emphasis is likely to be on forestry

for amenity use. More hardwoods are likely to be planted than conifers, and existing forests will be promoted as recreational facilities. Indeed, the latter is already happening in a dramatic way.

The commission has also been involved in "plant a tree in '73" activities, including reclamation of 2 hectares of a derelict iron ore site in Cumberland, 6 hectares of coal tip at Kilsyth in Stirlingshire, and 12 hectares of derelict mining valley at Aberdare, Glamorgan.

It is perhaps ironic that these changes should be coming about at a time when world market conditions have caused a dramatic increase in softwood prices; demand for home grown sawlogs almost doubled in the last quarter of the year covered by the report and there is, according to the report, a "strong desire" among British wood-using industries to use the dependable home grown product wherever possible. But with Recreation Planning Officers appointed in most conservancies, a feasibility study underway investigating development of low cost tourist accommodation on commission land, and the general restructuring of the commission's activities the future of forestry in Britain is clearly going to be markedly different from its immediate past. It seems appropriate that the commission is making a move to a new headquarters, near Edinburgh, at this time; it will be installed there by the middle of 1975.



international news

FOR the second time in less than a month, the Atomic Energy Commission has been hit hard with a critique of its safeguards against the theft of weapons-grade nuclear materials. The latest attack is particularly damaging to the AEC's public defence of its safeguards because it comes not from acknowledged critics of nuclear power but from an internal AEC study by four AEC consultants and one AEC employee.

Released last week by Senator Abraham Ribicoff, in spite of strenuous objections from the AEC, the study concludes that the commission's safeguards are "entirely inadequate" to prevent ingredients of nuclear weapons being stolen from the United States nuclear power programme.

A central criticism is that diversion of weapons-grade material from the power programme poses a greater threat to the public than the risk of a nuclear accident, yet "the relevant regulations are far less stringent". The study goes on to note that "It is our strong feeling that the point of view adopted, the amount of effort expended, and the level of safety achieved in keeping special nuclear material out of the hands of unauthorised people is entirely out of proportion to the danger to the public involved".

The AEC's reaction to these charges, outlined in a statement hastily distributed by its press office, is that there is not much danger of diversion of weapons-grade material at present be-

More criticism of nuclear safeguards

Colin Norman, Washington

cause very little highly enriched uranium or plutonium is used to fuel the light water reactors which now form the basis of the nuclear power programme. But the commission added that it is "taking a hard look at the study to determine what additional measures should be taken to further strengthen the requirements to safeguard nuclear materials from theft".

The point is however, that towards the end of the 1970s light water reactors will probably be operating on a plutonium cycle, which means that large quantities of weapons-grade nuclear material will be shipped around the country as reactor fuel. And if the fast breeder reactor lives up to the AEC's expectations, by the end of the century the amount of nuclear material flowing through the power programme could be sufficient to manufacture a quarter of a million bombs a year.

The study first finds fault with the system for accounting for nuclear materials in the fuel cycle. "The uncertainties in the accumulated material balances of the atomic energy operation of the country already make it impossible to say that an explosive

mass has not been diverted, and if reliance is placed solely on material balance methods that statement will have to be expanded many, many-fold in the near future," the report says. Furthermore, the present system will not pick up the diversion of nuclear material quickly enough: "A skilled diverter [could] have constructed an explosive device before the system can reach a conclusion that diversion has taken place".

A major recommendation is that the federal government should establish a special security force to guard nuclear materials in transit and for "all protection functions which could require the use of force". The study also recommends that the commission should forge strong relationships with the Central Intelligence Agency and the Federal Bureau of Investigation to make sure that those agencies are devoting sufficient resources to the problem of nuclear theft.

The whole issue of nuclear safeguards is rapidly building into a potent weapon in the hands of nuclear critics in the United States, particularly in respect of plans to build a string of plutonium-producing fast breeder reactors around the country. In response to such criticisms, Representative Melvin Price, chairman of the powerful Joint Committee on Atomic Energy, announced last week that his committee has launched a special review of the matter and will be holding some hearings later this year.

AFTER years of debating the construction of nuclear power stations, a proposition always vetoed by officials in the Finance Ministry because overseas experts assured them that power generated by conventional plants would be far less expensive in the foreseeable future, the Israeli Government has decided to put all its eggs in the nuclear basket.

Minister of Commerce and Industry Haim Bar-Lev has announced that from 1980 onwards, every power station built will be nuclear. The 1970s will see the construction of Israel's last new conventional power plant and her first nuclear one with a power-generating capacity of 600 MW, expected to be completed by 1982, when total national capacity is expected to be 4,200 MW.

An international tender for construction of the nuclear power station

Israel's nuclear plan

Nechemia Meyers, Rehovot

will be issued later this year by the Israel Electric Corporation, which has been charged with construction of the plant; the Israel Atomic Energy Commission, which operates the country's two existing research reactors at Sorek and Dimona, will be responsible for ensuring stringent safety standards.

While some locally-made equipment will be used, reactors will almost certainly be bought from the United States, and a representative of General Electric, Asher Engler, has stated that his company is willing to provide the required enriched uranium for the first few years of operation but that future supplies of the strategically important material would depend on an agreement between the governments of

Israel and the United States. Uranium could be extracted from phosphates from the Negev Desert, using locally developed methods, but it would still have to be enriched abroad.

Israeli environmentalists say that if Israel must have atomic power stations, they should be located in the furthest depths of the Negev, but the parched southern half of Israel lacks the large quantities of water required by nuclear power facilities for cooling purposes.

Whatever the validity of their arguments, the environmentalists have little chance of influencing government policy on this project. The first nuclear power station will soon be going up, probably near the new Mediterranean port town of Ashdod. If all goes well, there will be seven more by the turn of the century.

Soviet Scientists adopt new ploy

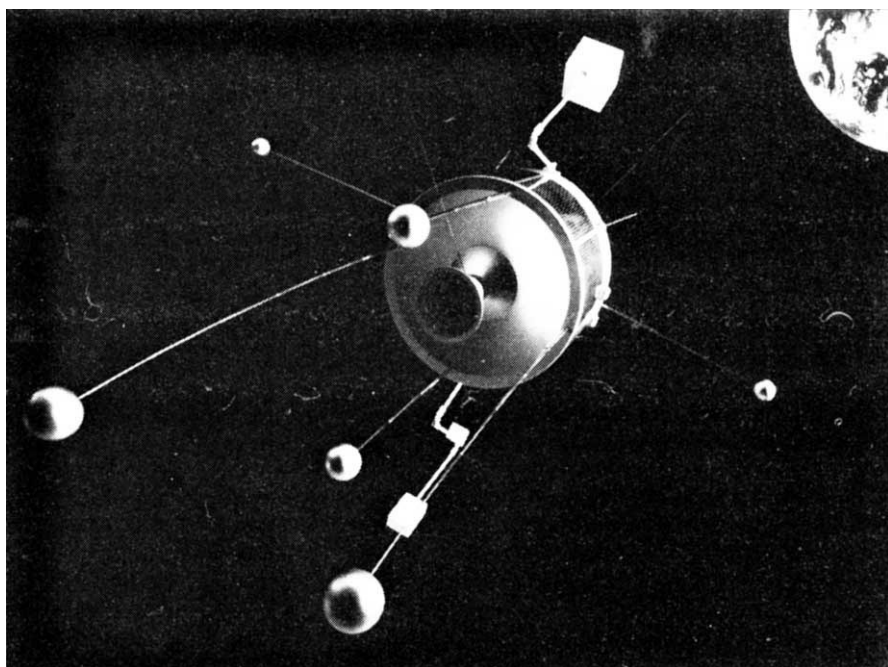
A UNIQUE international seminar will take place on July 1-5 in the Moscow apartment of Dr Alexander Voronel, a Soviet physicist who lost his job last year when he applied for a visa to emigrate to Israel. Organised by 19 Soviet Jewish scientists who have similarly been deprived of their jobs, and sponsored by an international board of advisors, the seminar is an attempt to keep the Soviet scientists in touch with developments in their own disciplines. It will undoubtedly pose something of a challenge to Soviet authorities.

Bearing the rather vague title of 'Collective phenomena and the applications of physics to other fields of science', the seminar has already met with considerable support in the international scientific community. A call for papers went out some seven weeks ago, and according to Dr Norman A. Chigier, professor of chemical engineering at the University of Sheffield, who is one of three international secretaries for the seminar, about 70 abstracts have so far been sent in.

Those who have submitted abstracts have been advised to apply for visas to enter the Soviet Union, and they have been told to make no secret of the fact that will be attending the seminar when they fill out their applications. Asked whether the Soviet authorities are expected to approve the visa applications, Chigier said that there is no way of knowing in advance, but that in the past they have been "unpredictable in their visa policies".

Chigier emphasised that the seminar should not be construed as "a confrontation with Soviet authorities". The Soviet Jewish scientists who are organising the event have been deprived of their right to travel abroad to attend scientific meetings and many have also been denied requests to attend meetings within the Soviet Union. According to Dr Edward Stern, professor of physics at the University of Washington, Seattle, who is also an international secretary for the seminar, the meeting has been organised to help Soviet Jewish scientists "break out of this scientific isolation which smothers and destroys scientific creativity." Chigier added that the event is "a gesture of solidarity".

All formal scientific meetings in the Soviet Union must be approved and organised by the USSR Academy of Sciences, but by labelling this event a seminar and holding it in a private apartment the organisers hope to circumvent the official regulations governing international scientific conferences.



GEOS: launching in 1976 to measure magnetospheric plasma and AC/DC fields.

The French in space

from the staff of La Recherche

THE relaunching of a European space policy, which should be confirmed by the creation as of mid-June of the European Space Agency (ESA), will give a second wind to French space efforts. The third space power, France has in fact launched but twelve satellites—only nine successfully—in twelve years of rather irregular activity. The enthusiasm of the first years was followed by an eclipse of three years (1965-67), then a stabilisation.

For the first time this year the French space budget will reach a thousand million francs. This is to be accompanied by a reorientation of the activities of CNES (the National Centre for Space Studies) towards greater European co-operation. The national programme becomes a support activity but bilateral operations, principally with the United States and the Soviet Union, will continue in order to permit participation in original programmes which are beyond French means. Thus scientific experiments will be put aboard the American probes OSO-1, Pioneer-Venus, IMP-L and Mariner-Jupiter-Saturne, while the Soviet Union will launch two small French technological satellites (Sret) and prepare the experiment EOS (release of a French balloon in the atmosphere of Venus). Co-operation with Germany will come about within a year with the launching of two telecommunications satellites (Symphony).

Thus France is putting its efforts into activities within the European community, in order especially to keep ahead of Germany. While continuing to participate in the programmes of

scientific satellites and applications devised by ESRO (European Space Research Organisation), such as the inhabited orbital laboratory Spacelab, Paris is in fact concentrating more on the heavy launcher Ariane. True to its policy of European independence, France considers that the independent exploitation of future European telecommunication satellites depends on having a launcher available, and has therefore decided to finance nearly 65% of the development cost of this launcher (2,472 million francs with 20% for contingencies) and to take control of the project. Technical and financial management has been assigned to CNES, under the control of ESA.

This is clearly an ambitious project and in taking over his post at the beginning of the year the new president of CNES, Mr Maurice Levy, recognised that it is "both a heavy responsibility and a technical challenge to which every resource will have to be devoted".

A three-stage launcher of 200 tons capable of placing 750 kg into fixed orbit. "Ariane" will be the largest rocket constructed in Europe and the first to use cryogenic ergols (liquid hydrogen and oxygen), which assure high performance but of which the Europeans do not yet have much experience.

The success of this project requires a combination of efficient management, thorough pilot studies and the maintenance in active service of teams and resources for French launchings. The launching range in Guiana will have an important part to play; up to 1977 it will have only six Diamant BP4 rockets to launch to put into orbit the

future French satellites Starlette (October 1974), Castor and Pollux (January 1975), D2B (mid-1975), D2B-Gamma (mid-1976) and Dialogue (end of 1977).

Thus the career of Diamant BP4 is practically finished and if a new 'dead period' is to be avoided before the arrival of the launcher Ariane, scheduled for 1980, a replacement must be found immediately.

Several projects are envisaged by CNES, for example a "Z launcher" which would use the two upper stages of Ariane and which could place 575 kg into low terrestrial orbit (400 km); the capability of Diamant BP4 is only 170 kg. But in fact the choice of the future national light launcher depends in part on an eventual programme of French military satellites and until now it has been uncertain whether this would make an entrance before 1980.

The electronic newspaper in view?

Ian Ridpath

WITH newsprint scarcities the world over, this could be an opportune time to turn into reality the science-fiction dream of an electronic newspaper that flashes information into each home at the touch of a button. And, as a result of a test programme currently under way, that is exactly what could be happening in the United Kingdom within two years.

These exciting plans stem from the realisation that almost every home has a data terminal for receiving visual transmissions—a television set. The broadcasting companies have decided to use this exciting facility to provide an extra service.

By coding the electronic newspaper transmissions on normal television broadcasts, and incorporating a decoder in domestic television receivers, the system can be run without using any extra band space on the already crowded airwaves. According to equipment manufacturers, the extra circuits needed in a new receiver would cost a thoroughly reasonable £20, although adding a converter to an existing set might cost between £50 and £100.

The trick in sending out the data is to use two television scan lines that are normally redundant. These are lines 17 and 18, which remain out of sight at the top of the picture. Each of these lines now contains pulsed information for one 'row' of the electronic newspaper's 'page'. A page is a screenful of data; there are 24 rows on each page and each row has 40 characters. Prototype receivers to pick up this year's forthcoming test transmissions are now being produced by industry.

A newspaper of around 60 pages will be produced at first, although up to 99

pages are possible. The complete set of pages will take less than 30 seconds to build up inside the receiver's store and continual changes will be added, second by second, as the news comes in. An editorial team at the transmitting centre will feed news into a computer, which will encode it for transmission.

This service would be particularly valuable at times such as elections, but for normal use the electronic newspaper is likely to carry sports results, stock market prices, weather reports, and news bulletins. Simple line illustrations will be possible on the system, and the independent channels may well include advertising.

It is difficult to foresee how sales of printed newspapers will be affected by electronic data transmission to the home. Existing radio and television coverage has not destroyed the newspaper market and the summarised information of the electronic newspaper may even increase demand for longer printed reports. But the potential of the system may have more subtle long term effects and there is clearly considerable room for lobbying by interested parties.

At home, the viewer with a suitable receiver will be able to watch television programmes normally until he decides on a change. His set will have a push-button console on which he can punch up the page number of the electronic newspaper he wants. Page 1 of the paper will probably be given over to an index of the full contents. The pages of the paper, which have been stored in the receiver, will then be displayed with a header line showing the time to the nearest second, thus increasing the effect of 'instant information'.

Both the BBC and the IBA (Independent Broadcasting Authority) independently developed their own electronic newspaper transmission systems, which they have been experimenting with since last year. But they have just agreed on an improved common system, engineering details of which are about to be published.

This standard system, which the prototype sets are designed to receive, enables viewers to pick up transmissions on all channels. Test transmissions to prove the new system's potential are to begin in the autumn, although it is not clear whether members of the public will be able to join in the fun of watching the editorial teams iron out their snags.

What is certain is that, after the trial period, a decision will be needed by the broadcasting companies, the receiver manufacturers and the Ministry of Posts and Telecommunications to begin full scale public services. It will be a historic decision, for this will be the world's first commercially available system of its kind.

Drug research in the UK

Peter Newmark

A SERIOUS failing of the drug companies was recently emphasised by Dr J. W. Black in his inaugural lecture as professor of pharmacology at University College, London. Returning to academic life after 15 distinguished years with ICI and Smith, Kline and French, he pointed out that industry was primarily interested in research and development of those drugs that might cure the most common diseases. This makes economic sense to the companies but means that those who suffer from rarer complaints are being cheated of their chance of a cure. The victims of, for example, multiple sclerosis and muscular dystrophy are included in this deprived category.

Professor Black offers a two-pronged approach to the alleviation of this imbalance. First, he is instigating a novel degree course aimed at overcoming the deficiencies and hence the paucity of academic, pharmacological research. Second, he suggests a National Drug Research Centre to concentrate on those areas neglected by industry. Where the vast funds for this would come from is anybody's guess. It is a sobering thought that of many thousand folic acid antagonists synthesised and investigated to date, only one, methotrexate, offers much to the treatment of cancer. And for those with the rarer disease it is only an inapt comfort that can be sought in the vast array of money-making antidepressants and tranquillisers that pour from the laboratories of the drug companies.

Latest figures compiled by the Office of Health Economics in London show that expenditure on medical research in the United Kingdom topped the £100 million mark for the first time in the financial year 1972-73. This represented an increase of about one-fifth over the previous year, though must be considerably less in real spending terms. Of the total expenditure, the government contributed 61%, charities and trusts a notable 13% and the pharmaceutical industry a dwindling 26%.

These statistics give no indication of which areas of medical research benefited most from the funds, let alone what returns were received by the investors, be they the public or shareholders. One thing that is certain is that most pharmaceutical research was done by the relevant industry. This is probably as it should be. To what extent it is in the public interest is less certain and the present government is not averse to nationalising parts of the industry if it feels that this is the best action for the nation.

THE more one enquires precisely how much meaning can be attached to a company's 'profit' as presented in its annual report, the more the answer seems to be 'relatively little'. This was highlighted in a paper by Parker and Gibbs presented recently to the Institute of Actuaries in London.

The drift of their paper is that companies of most kinds, except those in service industries, would come up with considerably smaller profits if inflation were not taken into account in some way or another. For example, it is conventional accounting practice not to take into account inflation when amounts are set aside for depreciation of plant and machinery, so the profit seems to be that much larger and has to be dipped into to replace that plant or machinery at the inflated prices. Likewise, "profits are overstated by the inclusion of profits on stock which arise solely from a general increase in price levels".

Parker and Gibbs produce some interesting findings using the CPP (current purchasing power) method of accounting. Here allowance is made for inflation by using a single index, for example the retail price index, to set up the company's accounts in terms of end-of-accounting-year pounds. This is a rather crude method, however, for it does not take into account the rise

Business report : profit and tax

Roger Woodham

in price of a particular commodity, which may be much more than the increase in a broadly based index.

Parker and Gibbs show that the chemicals sector would have earnings 15% down on average, oil 25% down, and general engineering and electricals 50% down according to CPP accounting. More specifically, ICI's earnings for 1972 would have been 45% less and BP's 65% less. In 1973 BP earned £310 million (compared with £70 million in 1972) but on huge total sales of £4,500 million. Only detailed analysis will reveal what inflation accounting would have made of that.

As J. M. Keynes is thought to have said: "It is better to vaguely right than precisely wrong".

● If the United States Internal Revenue Service (IRS) gets its way about the taxing of multinational companies based in that country, some companies may find it financially worthwhile to shift more of their research and development effort abroad.

Even the IRS admits that the situation is complex, but basically what happens now is that tax calculations for multinationals in the United States do not take into account the fact that some earnings outside the United States are generated by money spent in the United States, for example on research and development.

A company can obtain 'foreign tax credits' (which can be offset against United States tax) in respect of taxes paid to foreign governments. The IRS wants a proportion of 'home' expenditure on research and development to be shifted on paper to foreign operations, which means less foreign income and less foreign tax credits in the United States. The IRS has a complex way of calculating these credits and, in the final analysis, the pre-tax profits of multinationals based in the United States would be reduced—some say by 3%, typically. Certain companies, notably in the pharmaceutical industry, stand to lose more because their research and development programmes are particularly centralised; they would face a great pressure to do more research abroad.

The matter is still under consideration at the IRS and a decision is not expected for some months. The interested companies are, however, putting up a formidable legal fight.

Microscopic appeal

David Davies

A microscope as old as the society



THE Royal Microscopical Society has launched an appeal for £50,000 to expand its operations. In particular, it plans to increase its educational activities and publish its *Journal of Microscopy* more frequently. The appeal was launched by retiring President, Professor A. G. E. Pearse on April 30.

The society has had a chequered career. It was founded in 1839 when the microscope was as much as anything the preserve of amateurs but when exciting times were just around the corner for microscopists. Leeuwenhoek's seventeenth century resolving power of 1.25 μm had barely been improved on but the work of J. J. Lister on achromatic objective lens systems was just beginning to open up new horizons. By 1880 the resolution of the best microscopes was down to 0.2 μm and the society's aim to introduce and improve the microscope 'as a scientific instrument' began to be achieved.

Communication amongst microscopists depends very much on quality reproduction but the society was hardly in the forefront of publishing photomicrographs. It had published its first in 1853 (the ubiquitous proboscis of the fly) but it was not until 1906 that the *Journal's* lithographs began to be supplanted by photographs.

After 1890 there was little fundamental technical development possible for the optical microscope and the society went into a long period of decline. "Hibernation" one of its officers says, "is too mild a word for it". The question is whether the society has now been able to catch up with the lost opportunities of the dormant period and whether it will be able in the future to exert influence in the development of microscopy—now, of course, including electron microscopy. Many of the educational activities such as teacher training, provision of a technician's qualification and the publishing of teaching manuals, the bread and butter of many professional societies, are only now being planned. Another field that one fellow described as needing urgent attention is the "abysmally low" general standard of photomicrograph reproduction in books. Yet another problem is that a certain introversion and insouciance seem to possess, perhaps feeling their profession is merely a service industry.

The society has a hard pull ahead of it with much unglamorous work to do in establishing educational programmes. If it fails in its appeal, it will revert to a journal-producing and symposium - organising society, and science will suffer thereby.

Computers and social options

from the staff of *La Recherche*

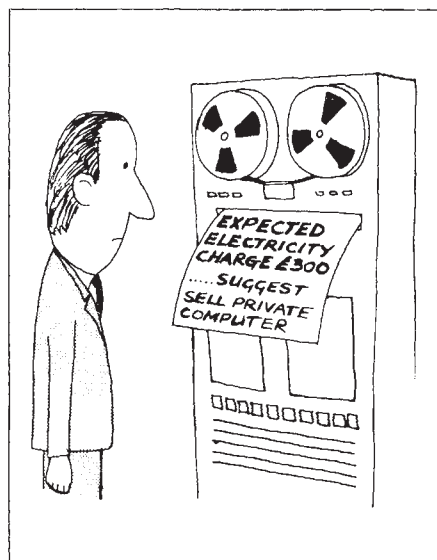
FOR the first time at the international level, information scientists and computer architects, sociologists and trade union representatives met earlier this month in Vienna to try to cope with the nagging issue of "Computers and Human Choice". Organised under the auspices of the 35-nation International Federation for Information Processing (IFIP) and the Austrian Cybernetic Society, the conference nicely complemented a symposium held two months earlier in Amsterdam, when the International Federation for Automatic Control pondered the problem of "Productivity and Man". Both meetings are clear evidence of the concern shown by professional societies for how today's machines are amplifying human mind and muscle and where the mechanical surrogate leaves man.

The job which the IFIP has set for itself is a big one: in the words of its president, Heinz Zemanek, it is "to stress the social aspects of the computer—to re-establish the human being as the centre of our existence". Large scale information systems have been motivated, to date, largely by industrial and commercial needs. (The military compulsions were not treated by the conferees at Vienna.) Harold Sackman (RAND Corporation) estimated, for example, that the public treasury of the United States has spent about \$100 thousand million thus far "on the development of computer services of all kinds, and the rest of the world has spent probably the same amount. But the individual citizen is the last to see a direct, useful return on his investment." Or, as expressed more effectively by John M. Carroll (University of Western Ontario), "the computer may be regarded by some as a repressive instrument which serves the causes of governments, corporations and large institutions. The common man too often sees himself as billed by them, taxed, enumerated, administered, manipulated, controlled—creased, folded, spindled and mutilated."

There is hope, nonetheless, that the social options open to the wizards of computing will prove to be more humane in the years to come. Citing a recent report by the Japan Computer Usage Analysis Institute, Alfred Dalling of the Austrian trade union of white collar workers reminded the meeting of the following time spread noted by the Japanese in the application of large scale information handling systems: 1945–70, 'big science,' 1955–80, the management base, and 1980–2000, the individual base.

With this projection in mind Ulrich

Briefs (West German Trade Union Federation at Dusseldorf) noted that one of the key problems in the long range strategy of organised labour is "whether it is better to fight for an improvement of the situation of employees . . . with no involvement in management decisions or . . . it is more advantageous to participate actively and cooperatively." In other words, does the noninvolvement of the employee in the management process lead to worker alienation? Kristen Nygaard, a computer scientist who is also an executive of Norway's labour union federation, could foresee that trade unions would help improve system design as labour becomes better informed, "as it acquires more information and, therefore, more power in the management-worker relationship. I could not see the unions in my country," he added, "approving the installation of visual terminals on



the shopfloor unless these were two-way links."

That the computer is not devoted principally to inventory and marketing problems or to payroll and insurance calculations was evidenced by some of the extensive (and impressive) social applications found for the computer in different geographical and ideological settings. G. R. Waddell, computer comptroller of the Edinburgh Corporation, explained the enormously complex base of accounting, personnel and multiple urban data comprising the municipal management information system (MIS) required to run the city of Edinburgh. Carroll described the experience in London (Ontario) of installing a "people's computer as an aid to democracy", of providing "the common man with easy access to computer facilities to even up the social, economic and political balance of power" in the form of a nongovernmental urban information service in that community. And Burt Nanus, director of the Center for Futures

Research at the University of South California, recalled that a Delphi study made for the American Federation of Information Processing Societies showed how MIS could "strengthen multinational public enterprises in such areas as [education] public health, criminal justice, pollution control, weather forecasting and disaster control, with major impacts before 1985".

An area of principal concern to the IFIP colloquium was that of fair information practices. Bo Hedberg (International Institute of Management, Berlin) emphasised that "we are approaching an information-rich society". System design of the means to process these data usefully, Hedberg continued, "is concerned with the distribution of power, implicitly or explicitly". Willis Ware, an engineer at RAND, proposed the creation by legislative action of a "code of fair information practice or perhaps an Informational Bill of Rights" in an effort to define, nationally then internationally, what is fair behaviour in this realm, "establishing safeguards and deterring deviation by judicial process and appropriate penalties". The IFIP agreed that it will examine the optimal methods, through an *ad hoc* computers-and-society committee, in order to deal with this and similar issues raised during the week-long deliberations.

Although some expressed the apprehension that "the electronic world is passing us by as we sit here debating—Nero fiddling while Rome burns," the last and far from pessimistic word on the subject went to the venerable, Russian-born physicist Lew Kowarski (CERN). He underlined his trust in man's use of the computer when he declared that the latter, "while it will relieve us of burdens, could also relieve us of all that makes life worth living. Before I count on the unforeseen—and therein lies our hope, albeit a pious one. The danger in computers comes not from the experts, but from the profiteers."

Australian answer

Peter Pockley, Sydney

AFTER studies by various Australian government departments, the Russian request to set up a joint facility "for photographing space objects and studies of the upper atmosphere," reported recently in *Nature*, has been rejected. The announcement was made on the same day that Prime Minister Whitlam called a snap general election. In the election atmosphere, the Labour Party could not afford the predictable attacks on grounds of favouring communists above capitalists, even if the scientific arguments in favour of the facility had been sound.

news and views

Maternal transmission of hepatitis

INTEREST in the transmission of viral hepatitis from the mother to her offspring is not new. The effects of hepatitis on the course of pregnancy and on the foetus have been studied since the report by Saint-Vel in 1862 on an epidemic of severe hepatitis in pregnant women in Martinique (*Gaz. Hôp. civ. milit. Paris*, **35**, 538; 1862). The world literature contains scanty and incomplete data and numerous divergent views. Stokes and associates (*J. Amer. med. Ass.*, **514**, 1059; 1954) presented evidence of 'silent carriage' of hepatitis B virus, and it is relevant to examine this paper in detail now that specific laboratory tests are available for hepatitis B and the problem of maternal transmission of this infection is being actively investigated.

One carrier described by Stokes was known to have transmitted hepatitis to at least four out of twelve recipients of his blood over a period of 3 years. His blood was also administered to four male volunteers, one of whom developed hepatitis. The second carrier is of particular interest. She was the mother of an infant who developed hepatitis at the age of 2 months. Volunteers given serum from her and her infant developed hepatitis. The third carrier was known to have transmitted hepatitis to four recipients of his blood, including one volunteer. The impact of apparently healthy carriers of hepatitis B virus on blood transfusion services and indeed on every aspect of medical practice is generally recognised. The problem of maternal transmission is just beginning to emerge.

Turner and colleagues (*Archs Dis. Childh.*, **46**, 616; 1971) reported the case of a young man who contracted hepatitis B after injection with an unsterile syringe. His wife, who was pregnant, developed the infection some 9 weeks later and her baby was born prematurely. After 59 days hepatitis B antigen was detected in the child's serum and antigenaemia had persisted so far through the first 2 years of life. The child remained clinically well but with some biochemical evidence of liver damage. Wright and associates (*Brit. med. J.*, **4**, 719; 1970) previously described the clinical history of a young dental assistant who developed jaundice late during her pregnancy. She was delivered of a normal female infant who, in turn, became jaundiced at the age of 5 months. Hepatitis B antigen was detected repeatedly in the infant's serum and in the mother's serum tested when the infant was admitted to hospital. Liver biopsy of the infant at the age of a year revealed chronic active hepatitis and cirrhosis. The mother, on the other hand, had remained symptom-free and her liver function tests were normal. Ohbayashi and colleagues (*Gastroenterology*, **62**, 618; 1972) tested sera collected in Japan from fifty-four members of three families with chronic liver disease or primary liver cancer. Hepatitis B antigen was detected in fourteen of fifteen affected members of the families and their siblings and in twenty of the twenty-four children of the female siblings. By contrast, the antigen was detected in only one of the eight children of the male siblings, and the antigen was found in the serum of his mother.

The finding of the antigen in these three families coincided remarkably well with the distribution of severe

chronic liver damage or primary liver cancer in the family tree. Familial clustering of cirrhosis has been reported in ten families in Japan since 1963. Of the thirty-nine affected members of these families, eleven died of cirrhosis and nine died of cirrhosis and liver cancer. It is notable that successive occurrence of liver disease for two generations was recognised in seven of the ten families, and the mother or her siblings were affected in each family. The next report by Merrill and her colleagues (*New Engl. J. Med.*, **287**, 1280; 1972) illustrates the other end of the spectrum. Of five children born to mothers who had hepatitis B infection at the time of delivery, four acquired the antigen 6–12 weeks after birth, but they remained clinically well and fulfilled the criteria for symptomless carriers. The fifth mother-child case is of interest in that the infant had hepatitis B antibody in his serum at birth (as did his mother) and he did not acquire the antigen.

Schweitzer and colleagues (*Gastroenterology*, **65**, 227; 1973) investigated some of the factors influencing neonatal infection by hepatitis B virus. A striking difference in the frequency of infection was noted between infants of mothers who had acute hepatitis B and those who were symptomless carriers of the antigen. Thirteen babies of twenty-seven mothers with the acute disease acquired persistent antigenaemia, especially if the maternal illness occurred near the time of delivery. By contrast, only one of twenty-one symptomless maternal carriers had a child who became antigen positive. The antigen was detected by radioimmunoassay in nearly half of twenty-one umbilical cord sera tested. The placental barrier to the antigen did not seem to differ in the acutely infected mother from that in the healthy carrier, and the presence of antigen in the cord blood was not necessarily related to subsequent acquisition of the antigen by the infant. In another report, Schweitzer *et al.* (*Amer. J. Med.*, **55**, 762; 1973) noted that the frequency of transmission of hepatitis B from mother to infant was high (76%) when acute hepatitis B occurred during the third trimester of pregnancy or early in the postpartum period, and low (10%) when hepatitis occurred during the first 6 months of pregnancy. Acute hepatitis developed in two of seventeen infants and the illness in both was mild, antigenaemia was transient and the homologous antibody developed during convalescence. No clinical illness was observed in thirteen infants (two infants were lost to the survey), but the antigen persisted in their serum accompanied by prolonged elevation of an enzyme frequently associated with liver damage. Liver biopsy performed on ten infants revealed this histological features of unresolved hepatitis and increased portal fibrosis was present in one infant. On electron microscopy, virus-like particles were found in the nuclei of six out of nine antigen-positive infants examined. Dunn and colleagues (*Exp. molec. Path.*, **19**, 113; 1973) found virus-like particles in explant cultures prepared from the remaining portions of some of these biopsies and they concluded that the particles proliferated within the nuclei of the liver cells.

In a more recent study on maternal transmission of hepatitis, Mazzur *et al.* (*Nature*, **274**, 41; 1974) determined the subtypes of hepatitis B antigen in 174 positive sera collected together with pedigrees in eighteen villages in south central Bougainville, an island in Oceania. Two different combinations of antigens were segregating in eleven out of thirty-two families tested, indicating that

these carriers had not been infected by each other nor from the same source. In families where the mother was a carrier, however, the twenty-two children who had anti-gaemia had, with a single exception, the same subtype as their mother. Among the twelve families where the mother was positive only one had mixed subtypes; while ten out of twenty families in which the mother did not carry the antigen had antigen of different subtype. Among six families in which the father was positive, mixed subtypes were found in four of the families.

The data thus suggest that vertical transmission from mothers to their children occurs, and also that horizontal transmission clearly takes place. Although vertical transmission and perinatal transmission from mother to child occur, the relative importance of each mode of transmission is not yet determined.

The significance of hepatitis B infection in early life lies not only in its relation to liver disease but also in its importance in the genesis of prolonged carriage of hepatitis B virus. Zuckerman and Taylor (*Nature*, **223**, 81; 1969) described a well-documented healthy former blood donor carrying hepatitis B antigen for at least 20 years. That a reservoir of chronic carriers may become established among children is, therefore, a cause for the utmost concern to the community. Methods for passive immunisation of susceptible newborn infants are already under evaluation, but the need for safe and effective vaccines is pressing. As a corollary the serum of pregnant women should now be routinely examined for hepatitis B antigen.

ARIE J. ZUCKERMAN

More to muscle than myosin

IN ways not anticipated, myogenesis has yoked in an uneasy alliance muscle biochemistry, molecular genetics and cell differentiation. The finding of Ishikawa and colleagues (*J. Cell Biol.*, **43**, 312; 1969) that arrow-head complexes—the *sine qua non* of actin—can be induced by H-meromyosin in the cortical regions of cartilage, nerve and intestinal epithelial cells, as well as in many kinds of embryonic cells, has been confirmed in many laboratories. Adelstein and colleagues (*Proc. natn. Acad. Sci. U.S.A.*, **69**, 3693; 1972) and others have found myosin in platelets, fibroblasts and nerve cells. Tropomyosin, the third of the contractile protein triumvirate, has also been found in several cell types (Cohen and Cohen, *J. molec. Biol.*, **68**, 383; 1972). Clearly many kinds of cell synthesise molecules identical or similar to actin, myosin and tropomyosin—molecules not long ago thought to be unique to terminally differentiated muscle cells.

Such results might satisfy the egos of muscle biochemists, but perplex those attempting to understand cell differentiation. Current models assume that the differentiated state is based on the activity of different sets of genes in different kinds of cells. A possible resolution of the dilemma of ubiquitous contractile proteins, and one with considerable theoretical implications for other aspects of differentiation, is the notion that there are several structural genes for different myosins and different tropomyosins, and that in different kinds of cell different myosin and tropomyosin genes are transcribed. Consistent with this scheme is the finding that the myosin light chains and the LMM portion of the myosin rod are products of different structural genes in fast, slow, smooth and cardiac muscles. Possibly during the differentiation of fast muscle, only those structural genes for fast myosin and fast tropomyosin become available for transcription and translation, whereas during the differentiation of platelets or cartilage cells two other sets of genes

If at first you don't succeed . . .

RADIOASTRONOMERS are about to begin another programme of 'listening' for signals from intelligent life within our Galaxy. Since the days of the original Project Ozma a decade and a half ago, radio observing equipment and techniques have improved and the rationale behind such a search has become more sophisticated. Previous efforts chiefly concentrated on hydrogen frequencies, since hydrogen is the most abundant element in the Universe. But recently there has been a growing feeling that any really intelligent race would probably preserve just these frequencies as free from artificial noise, because of their importance to radioastronomers. So the new investigation, which begins on May 8, will be using frequencies appropriate to emission from water.

The rationale behind this is partly the importance of water to life as human beings know it. In the wild, creatures meet at the water hole, so why not try the same sort of thing for contact between ourselves and extraterrestrial creatures? The frequencies are also ones which have been found in natural astronomical sources, notably clouds of gas and dust believed to be associated with young, forming stars. They are not expected to occur naturally in the region of older stars, which improves the likelihood that any such 'signal' from near such a star is a sign of life.

The astronomers responsible for this particular investigation are P. Feldman, of York University, Ontario, and A. Bridle of Queen's University, Kingston, Ontario; their programme involves the 150-foot Algonquin telescope, and is a "passive" one—Bridle is reported as saying that "we would not be capable of sending a signal our own equipment could detect over interstellar distances". Some six stars will be studied intensively from time to time (the first observing period devoted to the programme is 5 days) and 300 to 500 stars will be looked at more briefly over a year or two. The targets have been chosen on the basis that they are non-variable, slowly rotating (and so likely to have planets) and middle-aged (so that intelligence has had time to evolve).

News of this project arouses mixed feelings, since the 150-foot is a valuable instrument that might arguably be used for 'better things'—in the sense of studies which would definitely produce results. But 5 days here and there need be no great loss, and there will, of course, be no quibbling about the project if the North American team hit the jackpot.

JOHN GRIBBIN

for myosin and tropomyosin become available. This means that the genes for platelet or cartilage myosins are no more available in skeletal muscle cells than are the genes for albumin or pituitary hormone.

This ready solution, regrettably, is not as likely to explain the role of actin in the developmental programmes of virtually all cells. The issue with this strikingly conservative protein is whether the products of a single structural gene can be coupled to different kinds of myosins and tropomyosins, or whether there are several identical actin genes possibly even in different chromosomes participating in different developmental programmes. Circumstantial evidence for the latter stems from experiments showing that bromodeoxyuridine (BUdR)-suppressed myogenic cells

synthesise actin destined for the cell surface, whereas actin for thin filaments does not accumulate.

These considerations require re-evaluation of reports of the time during myogenesis when the messenger RNAs for myosin are transcribed and translated. If the presence of a molecule with the molecular weight of 200,000 is taken as evidence for the synthesis of myosin (Yaffe and Dym, *Cold Spring Harb. Symp. quant. Biol.*, **37**, 543; 1972) then all cells, including replicating presumptive myoblasts, must be transcribing and translating mRNAs for some kind of myosin. Accordingly it is not surprising that Rubinstein and colleagues (*Biochem. biophys. Res. Commun.*, **57**, 438; 1974) now report that myosin and actin are indeed coordinately synthesised and turned over, not only in myotubes but also in mononucleated presumptive myoblasts, cartilage cells, fibroblasts and BUdR-suppressed myogenic cells.

Lastly, a smouldering controversy of considerable interest to molecular geneticists exists between those who claim that myoblasts withdraw from the cell cycle as a precondition to fusion, and those who claim that cessation of DNA synthesis results from fusion. The most emphatic statement of the second position is that of Doering and Fischman (*Devl Biol.*, **36**, 225; 1974). Based on what is termed a "% fusion" index they conclude that myogenic cells whose DNA synthesis is blocked by Ara-C fuse; according to these authors replicating myogenic cells have the option to replicate, or to fuse, and, as a consequence of fusing, cease replicating. Before these important conclusions can be accepted, however, it should be noted that: (1) Ara-C is routinely used to kill replicating cells, so that the design of the Doering and Fischman experiment must yield a spuriously high "% fusion" index; (2) early fusion cannot be measured with the light microscope; and (3) it is impossible to distinguish 'fibroblasts' from presumptive myoblasts. Given these uncertainties it will be interesting to see if those who speculate that myoblasts withdrew from the cell cycle as a precondition to fusion can devise more cogent experiments.

H. HOLTZER

Slaved disk model for Hercules X-1

from James Pringle

A POSSIBLE solution to some of the more puzzling aspects of the X-ray star Hercules X-1 has been proposed in a recent article by Roberts (*Astrophys. J.*, **187**, 575; 1974). The X rays from Her X-1 are regularly pulsed with a period

of 1.24 s and the source of X rays is one member of a binary star system which has an orbital period of 1.7 d. The X-ray source is thought to be a neutron star with a strong dipole magnetic field and the X rays are produced by material transferred from the 'normal' star of the binary system accreting on to the compact neutron star. Because of the strong magnetic field, the accreting material is channelled down on to the magnetic polar caps of the neutron star dissipating its kinetic energy of infall when it strikes the surface. So much energy is liberated in such a small region that the temperature rises to more than 10^7 K. Thus most of the radiation emerges as X rays. The two magnetic polar caps are then two X-ray emitting hotspots: if the magnetic axis is offset from the rotation axis, rotation of the star with a 1.24 s period provides a natural explanation of why the observed X rays are regularly pulsed.

Furthermore, the X-ray source displays a third periodicity. The X rays are visible for only about 10 to 12 consecutive days, reappearing regularly at 35-d intervals. Explanations of this phenomenon—for example, variable mass transfer or precession of the pulsing neutron star—have difficulty in accounting for another aspect of the problem. The 'normal' component of the binary system was identified in 1972 as the previously known variable star HZ Herculis. This star is so close to the luminous X-ray source that X-ray heating causes one side of it to be about four times brighter than the other. The brightened face follows the neutron star around its orbit, and thus HZ Her can be observed to vary regularly in brightness with a period of 1.7 d. The puzzling feature of this 1.7-d optical variation is that, although the X rays can be seen for only 10 out of every 35 d, the heating of the side of HZ Her nearest to the X-ray source continues throughout the cycle, even when no X rays are observed.

Roberts' explanation of this is quite simple. He suggests that HZ Her has its rotation axis at an angle to the orbital rotation axis. In this case, the rotation axis of HZ Her precesses about the orbital rotation axis with a period of about 35 d. The material transferred by HZ Her towards its companion has too much angular momentum to be able to fall on to it directly and, instead, forms an accretion disk around the neutron star. In the disk, viscosity transfers angular momentum outwards, allowing the material to spiral slowly inwards. Roberts notes that the disk, which is opaque to X rays, will lie in the plane perpendicular to HZ Her's rotation axis, and thus in general not in the orbital plane of the system. Roberts names this the Slaved Oriented

Disk, or SOD for short. Thus the angle between our line of sight to the X-ray source and the plane of the SOD varies with the 35-d precession period of HZ Her. Since the X-ray source can only be seen when this angle is sufficiently large, one expects the visibility of the X rays to vary with the 35-d period. On the other hand, HZ Her is so close to the X-ray source that the (X-ray) shadow cast on its surface by the disk is quite small. Thus one expects the heating of HZ Her to continue, even when the disk temporarily obscures the X-ray source from observation.

This is by no means the whole story. Roberts also explains why the rotation axes might be expected to be misaligned and shows how his model can account for several further observational details. There are now a number of models which seem to account for the 35-d period of Her X-1 and its associated problems, but nobody has yet performed any detailed calculations. Roberts, however, excuses himself by quoting J. A. Wheeler's First Moral Principle: "Never do a calculation until you already know the answer".

Asia as a composite continent

from Peter J. Smith
Geomagnetism Correspondent

WHETHER or not it is realistic to suppose that any of the present continents could have formed by the accretion of separate crustal blocks, the important question of continental fusion continues to receive considerable attention. Most recently the debate has centred particularly on Africa (see, for example, Piper *et al.*, *Nature*, **245**, 244; 1973) with new evidence suggesting that the orogenic belts between the major cratonic areas of that continent may not be sutural but more probably formed *in situ*, although the idea of what Kropotkin (*Eos*, **53**, 180; 1972) has termed the "composite continent" was actually first applied to Asia by Argand (*Coll. Rep. 13th Int. Geol. Congr. 1922*, **1**, 171; 1924) just 50 years ago and long before the new global tectonics had been thought of.

Since then, evidence supporting the composite nature of Asia has been adduced from palaeomagnetism, palaeontology, geology and petrotectonics. In a useful and commendably concise article Burrett (*Earth planet. Sci. Lett.*, **21**, 181; 1974) has now brought all these data together to delineate the Asian block boundaries and to estimate the timings of the various collisions thought to be involved. Although the field information is not entirely clear in all cases, there seem to be about nine blocks present; and Burrett sum-

marises the evidence for each.

The general conclusion that Burrett comes to is that the coincidence of orogenic belts containing zones of high pressure metamorphism, ophiolites and deep water sediments with faunal province boundaries only makes sense in plate tectonic terms, the belts being the sutures along which the crustal fragments collided and coalesced. He then goes on to discuss when these collisions took place, beginning with the assumption that in the Lower Palaeozoic the various blocks were separated by considerable widths of oceanic crust. According to this analysis the first collision took place in the Upper Carboniferous, although the fusion of all blocks into what is now Asia was not complete until well into the Mesozoic.

Burrett admits that his model is based on "very incomplete data" but nevertheless suggests that "there does not appear to be much room in which to alter the boundaries of the blocks here identified nor the timing of their collisions". It remains to be seen whether, as in the case of the African orogenic belts, a static model can be made to explain the data even more convincingly.

Sources of natural radiation

from J. R. A. Lakey

THE largest source of ionising radiation exposure to the United Kingdom population originates from terrestrial and cosmic radiation. This natural radiation was the subject of a meeting of the Society for Radiological Protection held on April 2. C. R. Hill (Institute of Cancer Research, Sutton) stressed that the exposure is by no means uniform. The living habits of human beings have a marked effect on the dose because of the natural radionuclides in the diet and the radioactivity of building materials. These differences have been exploited—in attempts to deduce a dose/effect relationship—but with little success because control populations are hard to find. Nevertheless there has been some pressure to use the apparently solid ground of natural radiation exposures as a basis for setting radiation protection standards. The International Commission on Radiological Protection has not made its recommendations on this basis but J. Vennart (Medical Research Council, Radiobiology Unit, Harwell) reminded the society that natural radiation is deliberately neglected and controls are applied only to artificial radiation exposure.

An interesting dilemma arises from the use of building materials which are naturally radioactive. G. A. M. Webb

(National Radiological Protection Board, Harwell) revealed that the Soviet Union has limits designed to avoid 'abnormal' radiation exposure and prohibits building materials containing more than 10 pico curies of radium-226 per gram. Most building materials are not significantly radioactive so that the house walls and foundation act as a shield against natural radiation exposure.

Several speakers referred to the more insidious radon-222 which is present in air at around 0.1 pico curies per litre and in some cases appears in drinking water at concentration as high as one million pico curies per litre. E. I. Hamilton (Institute of Marine Environmental Research, Plymouth) said that the apparently inexplicable variations in the activity of radon in drinking water occur because the gas can move through the crack system from rocks as deep as 3,000 m.

The dose from natural radiation has been reviewed extensively by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). F. W. Spiers (University of Leeds) discussed the dose due to internally deposited radioactivity, and said that doses reported by UNSCEAR for cortical and trabecular bone should be revised. The fat cells, which account for 53% of bone marrow, concentrate carbon-14 and the dose becomes four times higher than UNSCEAR reported. Surface-seeking alpha emitters also increase the dose to osteoblasts lining the cavities in bone. The genetic effect of natural radiation has been of most concern to human populations and it must account for some of the births subject to genetic disease. R. J. Berry (Royal Free Hospital, London) said that the radiation dose to double the mutation rate is thought to be between 30 to 80 roentgens. Nobody has been able to correlate natural radiation dose with genetic diseases and it is not necessarily correct to assume that all mutations are harmful. Indeed there is some evidence to suggest that some radiation-induced effects are repaired.

Sendai virus receptors in a model membrane

from a Correspondent

VERY little is known about the first step in replication of animal viruses—the absorption of the virus to receptors of the host cell. It is surprising, for instance, that the question of whether the small oncogenic DNA viruses bind to specific cell receptors is still unresolved. By contrast, the complementarity involved in the reaction of myxoviruses and paramyxoviruses, such



Electron micrograph of Sendai virus adsorbed to a ganglioside-containing liposome. The virus can be distinguished by the spikes on the outer surface. The length of the attachment of the two viruses is 2,100 and 2,500 Å. The bar represents 1,000 Å. (From Haywood, *J. molec. Biol.*, **83**, 427; 1974.)

as Sendai virus, with specific receptors, at least in the non-permissive erythrocyte, is well known. Haywood (*J. molec. Biol.*, **83**, 427-436; 1974) has extended this system to a study of the interaction of Sendai virus with a model membrane incorporating the specific receptors.

Sendai virus has a spiked appearance, the spikes being surface glycoproteins which absorb to sialic acid groups of the receptors of erythrocytes and presumably also of permissive host cells. Haywood now reports that sialic acid groups of gangliosides work well as receptors for Sendai virus if they are incorporated into synthetic lipid membranes at relatively high surface density. Electron micrographs show virus particles adsorbed to the liposomes containing ganglioside along long stretches (210 nm–250 nm) of the virion. Assuming the area of attachment to be a circle of this diameter, then it seems that the virion is absorbed to a considerable patch of membrane and makes multiple attachments between spike glycoproteins and clusters of gangliosides, perhaps as many as 3,000. It would be interesting to know if the clustering of receptor molecules is actually induced by binding to the virus. If so, then this process might be related to cell fusion induced by viruses such as Sendai, since high local concentrations of gangliosides destabilise lipid bilayers.

The absorption of Sendai virus to

liposomes is specific for structures containing gangliosides or a positive lipid such as stearylamine. The types of binding to these two sorts of liposomes are clearly differentiated, however, since only the membranes which incorporate the sialic groups can compete effectively for virus in mixture with erythrocytes. The weak nonspecific binding of Sendai virus to liposomes containing stearylamine is probably electrostatic since the virions have a net negative charge.

The way in which sialic acid groups are presented to the virus is of prime importance for high affinity binding. For example, very small liposomes containing gangliosides obtained by sonication are less potent inhibitors of viral haemagglutination than are liposomes with a greater total surface area. Second, ganglioside micelles do not compete with erythrocytes for virus. Probably both a threshold surface area and ganglioside density are needed in order that the virus can make a critical number of contacts with the absorbing surface. A somewhat similar interpretation probably explains the finding, made earlier by several groups, that the tightness of binding of myxoviruses to sialoglycoproteins depends critically on the molecular size and is drastically decreased by proteolysis of the receptor glycoproteins. The reason that a large number of contacts are required for firm attachment to receptors containing sialic acid might be explained if the bond energy between receptor groups and viral spike glycoproteins is low.

Whether gangliosides or sialoglycoproteins or both are the naturally important receptors for Sendai virus in cells which support a lytic infection is unknown. Haywood suggests that the probable topography of the cell surface is such that the first viral contacts may be with glycoproteins, after which virions are handed over to gangliosides situated nearer to the bilayer and in a better position to initiate events leading to penetration. Perhaps this process involves the viral neuraminidase in localised reactions which break the initial binding of virions to receptor glycoproteins and allow attachment to internal gangliosides to take place.

New advances in SEM

from a Correspondent

At the seventh Annual IIT Research Institute Symposium on Scanning Electron Microscopy in Chicago from April 7 to 11, probably the most significant advance in instrumentation was that reported by O. Wells and A. Broers (IBM Research Laboratories). These workers immersed the specimen in the high field region of the final lens

and collected the low energy loss electrons coming from the edge of a highly tilted specimen. They were then able to demonstrate centre/centre resolution of 3 nm and an edge resolution of 1.5 nm. The instrument used was the 100 Kv LaB₆ gun designed by Broers, who thought that the resolution could now only be further improved by using a single crystal of LaB₆ to replace the finely pointed sintered rod.

M. Isaacson (University of Chicago) presented a frank discussion of the problems associated with specimen preparation for the high resolution scanning transmission electron microscope presently under development in several laboratories. Although the instrumental potential now exists for atomic resolution, specimen contamination and beam damage represent real problems which must be solved before a wide range of biological macromolecules are going to be imaged in these new microscopes. Isaacson and his colleagues considered the answer to these problems lay in the use of LHe II stages, low atomic weight negative stains and computer image processing to remove background noise.

Several speakers discussed real-time stereo viewing and dimensional reconstruction of stereo pairs. A. Boyde (University College, London) outlined the problems associated with such reconstructions and demonstrated the usefulness of the method in analyses of complex tri-dimensional objects and in interpreting dynamic experiments in the microscope. But the most convincing demonstration of real-time stereo viewing was given by E. Chatfield and colleagues (Ontario Research Foundation). By using a single post lens deflection coil they took a dual signal which was displayed on a colour TV set utilising the red and green guns. The image could then be viewed using red and green separation filters in order to reconstitute the 3D image.

N. Barbi and colleagues (Prairie View, Illinois) gave some data on a windowless energy dispersive X-ray detector in which it was claimed that they are able to get significant signals for C, O and N though problems of contamination and beam collimation still remain. There were several comments on some of the ancillary techniques associated with SEM, including advances in cathodoluminescence, autoradiography and histochemistry. None, however, demonstrated the necessary breakthrough which would make these techniques of any significant use in scanning microscopy. In the biological sciences, J. Nowell (University of California at Davis) gave more details of the injection replication technique for internal cavities of animal tissue and W. Humphreys and colleagues (Uni-

versity of Georgia) outlined a useful variation on the critical point drying technique which enables cryofractured specimens to be visualised in the SEM.

Scanning electron microscopy seems to have come of age and there are now more than 25 different models of microscope from which to choose. The IITRI SEM symposia have played no small part in the growth of the subject and it will be of interest to see whether future meetings can continue to contribute to the requirements of scanning electron microscopists and not degenerate into a dreary catalogue of photographs of what can be seen using this instrument.

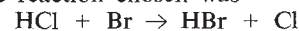
Activation energies under scrutiny

from our Chemical Physics Correspondent

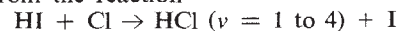
For many years discussions of thermal reaction rates have centred on the activation energies, E , of reactions whose rates have followed the Arrhenius expression, $k = A \exp(-E/RT)$ and its more modern variants. Its justification requires reagents to possess the energy E in order to surmount an energy barrier and proceed to reaction. The degrees of freedom holding the energy were considered irrelevant except that their number might influence the factor A . It has long been recognised that this assumption was not fully correct but experiments to show the specific influence of particular categories of initial energy have not been easy to devise.

In recent years a number of cases have been studied where the excess energy of the products of an exothermic reaction has resided in specific vibrational excitation. The principle of microscopic reversibility then requires that analogous reactions in the reverse direction should proceed preferentially with the reagents in excited vibrational states. It is just such a process which has been elegantly demonstrated by Douglas, Polanyi and Sloan at Toronto (*J. chem. Phys.*, **59**, 6679; 1973).

The reaction chosen was



for which the HCl reagent could be prepared in excited vibrational states from the reaction



Such a stream of vibrationally excited states was crossed with a stream of Br atoms which was pulsed on and off 15 times a second. The occupancy of the vibrational levels of the HCl was monitored through the infrared emission spectrum and the change between the on and off periods for the Br was simultaneously obtained by means of a phase sensitive detector set to 15 Hz. The frequency setting of the spectro-

meter monitoring the emission was swept and its resolution was such that each separate vibration-rotation state characterised by ν and J could be monitored. As expected it was found that whereas the levels $\nu = 1, 2, 3$ and 4 were easily seen in emission, the depletion of these levels by reaction with Br for $\nu =$ was greater than for $\nu = 3$, but changes in the populations of the levels $\nu = 2$ and 1 by reaction were scarcely detectable.

Although this result in itself is not surprising and leaves the principle of microscopic reversibility intact, the elegance, comparative simplicity and excellent sensitivity of the technique imply that its future sophistication will lead to considerable detailed experimental evidence about reaction energy surfaces. This should prove valuable in a field where theory has tended to out-run experiment.

Double repression of galatose genes

from Benjamin Lewin

Molecular Genetics Correspondent

THE interactions which control repressible and inducible operons in bacteria are now well understood in principle: in the lactose operon, for example, repressor protein, cyclic AMP receptor protein (activated by cyclic AMP) and RNA polymerase act at sites on DNA close to the structural genes under control. The galactose operon, however, is a more puzzling system than usual, and it has been difficult to envisage molecular mechanisms for its control. By characterising a new regulator mutation, Hua and Markovitz (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 507; 1974) have now identified a new control site in the operon, analysis of which may help define this system.

The galactose operon is controlled by three systems. A repressor protein (specified by the regulator gene *gal^R*) inhibits transcription in the absence of galactose by binding to an operator (defined by mutation) located at one end of the structural gene cluster. The cyclic AMP receptor protein and RNA polymerase interact at a site (presumed to be close to this operator) to initiate transcription. The third system of control establishes repression in response to the *cap^R* gene, which is part of a regulator system responsible for controlling synthesis of products required for formation of the capsular polysaccharide. Because mutations in the *gal^R* and *cap^R* genes each relieve only some of the repression of the operon, and appear to act independently, it has seemed likely that the galactose operon might possess two operators.

This hypothesis has now been con-

firmed by the results reported by Hua and Markovitz. Operator mutants lacking response to an active *gal^R* locus have in the past been isolated; and these identify the operator at which galactose repressor protein binds. By selecting cells which do not respond to an active *cap^R* gene, they obtained a mutant with the characteristics expected of an operator unable to respond to the *cap^R* product. The mutation is closely linked to the first structural gene of the operon.

Since the cyclic AMP system stimulates transcription *in vitro*, it seems very likely that, analogous to the lactose operon, it acts at a promotor adjacent to the galactose operator responsive to *gal^R* product. That the second operator, on which the *cap^R* product acts, may also be accompanied by a promotor, this one unresponsive to the cyclic AMP system, is suggested by the observation that bacteria deficient in cyclic AMP can grow on galactose. From the characteristic of mutants affecting expression of the galactose operon, Hua and Markovitz suggest that the *gal^R* responsive sites lie immediately adjacent to the structural genes, with the *cap^R* responsive sites lying just beyond them.

Obvious topological problems are raised by the presence of two operators in one operon. How does repression at one influence transcription from the other? Given present models for repressor-operator interactions, it is not immediately obvious how expression from the further control sites takes place when the inner sites are repressed. And when both sites are supporting transcription, one might expect changes in the state of DNA to occur as polymerases readthrough the inner sites—do these influence its activity?

How may these control systems be better characterised? One obvious experiment is to map the two operators more precisely to determine their order and distance apart. Also important is the necessity of characterising the promotor(s) by mutation, since their postulated properties have been inferred almost exclusively from biochemical experiments. It will be interesting to see whether the two operator and promotor sites are indeed each independent or instead represent overlapping sequences in one region of DNA. Isolation of the *cap^R* product is another urgent necessity, since this may permit experiments *in vitro* to characterise the mechanism of transcription. Even with such critical points yet to be elucidated, however, one important conclusion is clear: an operon may be controlled by more than one repressor protein and may have more than one operator sequence. This has obvious implications for control of gene action in eukaryotic cells.

Interplanetary dust on Earth

from Peter J. Smith

Geomagnetism Correspondent

EXTRATERRESTRIAL material captured by the Earth is generally believed to comprise mainly interplanetary dust, the more spectacular contribution from meteorites being relatively small. Unfortunately, however, not only is interplanetary dust much more difficult to identify than meteoritic debris, there are also likely to be problems in separating it from the terrestrial material in which it is highly diluted. The result is that the nature of most of the material reaching the Earth from outside remains largely unknown. On the other hand, there is no reason in principle why the products of interplanetary dust on Earth, if they exist, should not be identified. Wasson (*Icarus*, **2**, 54; 1963), for example, showed that the presence of extraterrestrial dust would be proved if radioactive nuclides produced by the action of high energy (5-100 MeV) solar protons on the dust in interplanetary space could be detected in terrestrial materials. It is true, of course, that most of these radionuclides may also be produced from spallation reactions induced by galactic cosmic rays; but although such processes are predominant in meteorites they are fortunately negligible in bodies smaller than about 1 cm.

The detection of extraterrestrial dust by radionuclides thus seems promising, although even here there is a practical problem in that some of the expected nuclides are also produced on Earth by other natural nuclear processes. ²⁶Al, for example, seems particularly attractive because of its high rate of production, its long half life (0.7×10^6 yr) and its β^+ radiation which is relatively easy to detect. But unfortunately it is also formed by spallation of atmospheric argon at a rate apparently comparable to that expected from processes involving interplanetary dust. The precise relative importance of terrestrial and extra terrestrial ²⁶Al is actually a matter of dispute; but as long as the dispute exists, convincing evidence for the presence of extraterrestrial dust is unlikely to be obtained from this particular nuclide.

The only two nuclides suitable from the points of view of production rate and half life and thought to have no significant natural production on Earth are ⁵³Mn and ⁵⁹Ni. Accordingly, Bibron *et al.* (*Earth planet. Sci. Lett.*, **21**, 109; 1974) have attempted to detect and measure ⁵³Mn in samples of snow from the central area of the Eastern Antarctic Plateau. This site was particularly suitable for the purpose because it is remote from marine, continental and

industrial sources of terrestrial contaminants. Moreover, the average annual temperature is -60°C and the maximum temperature never exceeds 0°C , so that materials precipitated are perfectly preserved and do not diffuse. There is also another important advantage. Although there are no known natural processes on Earth likely to give rise to ^{53}Mn , this nuclide could be produced as a result of thermonuclear bomb tests in the atmosphere. There is apparently no published data which can either confirm or refute this possibility; and so in order to avoid the problem altogether it is necessary to investigate only snow which fell prior to 1952. At the site in question the rate of snow accumulation has been extremely low, making it possible without too much difficulty to collect firm layers deposited well before nuclear testing began.

^{53}Mn was detected in all three samples of snow analysed (corresponding to the period 1935-1950), the average activity being 0.82 ± 0.17 disintegrations per minute per 10^3 tons of snow. This is apparently the first experimental evidence for the presence on Earth of the natural nuclide. But did it really originate from the supposed interplanetary dust? There is, in fact, another possibility—that the ^{53}Mn was produced not from the dust but from the material volatilised or dispersed from meteorites during their flight through the atmosphere. To account for the observed ^{53}Mn activity, however, the volatilisation of over 3×10^6 tons of chondritic material would have been required between 1935 and 1950 whereas, in spite of uncertainties in the estimates, it appears unlikely that the actual meteoritic influx exceeded 6×10^4 tons (or perhaps twice that, taking into account objects with pre-atmospheric masses of less than 10^3 g). In short, the meteoritic material available would have been insufficient by a factor of at least 25. Bibron and his colleagues thus conclude that the only possible source of the ^{53}Mn is indeed the interplanetary dust.

Accepting this conclusion they go on to estimate the total global influx of extraterrestrial dust. During the period 1935-1950 the rate of deposition of ^{53}Mn in Eastern Antarctica was $(2.2 \pm 0.5) \times 10^{-5}$ disint. $\text{min}^{-1} \text{m}^{-2} \text{yr}^{-1}$, although this is not a value which can be extrapolated directly to the whole Earth. It is probable that a significant proportion of the extraterrestrial material volatilises when it enters the atmosphere, becoming fixed on the stratospheric aerosols and thus ultimately being deposited in such a way that the concentration is maximum in temperate latitudes and minimum at the poles and the equator. The deposition in Antarctica should thus represent a lower limit of the average over the

Earth's surface. Assuming (in the absence of any more definite information) that the ^{53}Mn distributes itself in a way similar to that of the ^{90}Sr injected into the stratosphere by thermonuclear explosions, Bibron *et al.* estimate the average global deposition of ^{53}Mn to be $(6.6 \pm 1.5) \times 10^{-5}$ disint. $\text{min}^{-1} \text{m}^{-2} \text{yr}^{-1}$. From this figure it is then possible to show that the interplanetary dust (assumed to have a chondritic composition) accumulates on the Earth at a rate of 1.6×10^6 tons a year.

In summary, then, Bibron and his colleagues have provided the first incontestable (their word) evidence for the presence on Earth of ^{53}Mn , a radio-nuclide thought to be formed only from interplanetary dust which builds up on the Earth at a rate of about 10^5 tons a year. But this is only a beginning, for most of the questions about interplanetary dust remain unresolved. As Bibron *et al.* point out, a decisive step forward would be the simultaneous determination of ^{53}Mn , ^{59}Ni and ^{26}Al , although there is still plenty of scope in tracing the variations of ^{53}Mn itself during the past hundred thousand years or so by analysing deep ice cores from polar ice sheets.

Vegetational change in an aboriginal environment

from Peter D. Moore
Plant Ecology Correspondent

EVER since Iversen (*Danm. geol. Unders.*, 2R, 66, 1; 1941) described the influence of Neolithic cultures on the vegetation of Denmark, palaeoecologists have been forced to accept the fact that the introduction of agricultural practices in north-west Europe (perhaps 3,500 radiocarbon years BP for the British Isles) profoundly altered the contemporary environment. More recent work has suggested that many of the vegetational changes which were once thought to be climatically determined must now be regarded as anthropogenic, for example the decline in lime (*Tilia*) in Britain (Turner, *New Phytol.*, 61, 328; 1962) and the initiation of blanket peat formation (Moore, *Nature*, 241, 350; 1973). Some have even considered it possible that pre-agricultural Mesolithic cultures may have modified vegetation on a large scale by using fire to drive game or to favour selectively plant species which provide a source of food such as hazel, *Corylus avellana* (Rawitscher, *Nature*, 156, 302; 1945). This view has received some indirect support as the result of the recent work of Martin (*Aust. J. Bot.*, 21, 283; 1973) on the environment of aborigines in the Nullabor region of South Australia.

Martin's interest has centred on the palynological analysis of three radiocarbon-dated cave deposit profiles and their interpretation in the light of modern pollen deposition in the area. Her modern pollen studies have provided a means of recognising past plant communities which, in this arid region, are fairly simple in composition.

The present vegetation of the Nullabor region consists of mallee scrub in the coastal region, which has a slightly higher precipitation ($23\text{--}25 \text{ cm yr}^{-1}$) than the inland area ($16\text{--}18 \text{ cm yr}^{-1}$). Temperatures in the two regions are virtually the same, but the number of rain days in the coastal region is roughly twice that experienced inland (80 rather than 30-50 d inland). In the arid interior there is tall, open scrub dominated by *Acacia* and *Myoporum*.

Palynologically the two vegetation types can be separated on the basis of the ratio of Myrtaceae (largely *Eucalyptus* and *Melaleuca*) pollen to that of Chenopodiaceae (many genera including *Atriplex* and *Kochia*). Surface pollen analyses in the arid scrub region consistently showed Myrtaceae:Chenopodiaceae (M:C) ratios of less than 0.2. M:C ratios in samples from the less arid mallee scrub zone, on the other hand, were always in excess of 0.5. This simple characteristic of the pollen fallout from the two vegetation types permitted Martin to recognise these communities in the fossil pollen assemblages of the cave deposits.

At one site (Eucla) the M:C ratio increases from a low level at 20,000 BP to a maximum at the present day. Its rate of increase mirrors effectively the postglacial eustatic rise in sea level resulting from the gradual melting of the icecaps. The vegetational change in which arid scrub gives way to mallee can thus be explained by a rise in climatic wetness resulting from the increasing proximity of the coastline.

Two other sites tell a rather different story. They show an increasing abundance of mallee scrub (that is, a higher M:C ratio) until about 4,000 BP when it apparently declined. The date of this decline coincides with an increasing abundance of aboriginal artefacts at these sites. The aborigines of recent times are known to have burned scrub extensively, both for ritualistic purposes and to encourage certain food plants, such as the wild gooseberry. Under the climatic stress of aridity it is quite feasible that repeated burning of the scrub could have reversed the trend in vegetational development caused by ameliorating climate and have produced an anthropogenic plagioclimax of arid scrub. This seems to be one of the best documented examples of the influence of a pre-domestication human culture on the course of vegetational development in a wide geographic region.

European tumour virology at Aviemore

from Elizabeth Rogers

THE momentum of tumour virus research is such that pauses for breath are essential if one is not to be left gasping behind. So it was that an informal meeting of European tumour virologists at Aviemore, Inverness-shire from April 3–5 provided an opportunity for gathering together the threads of recent progress. The meeting was organised by Professor J. Subak-Sharpe (Institute of Virology, Glasgow) and the sixty or so short contributions covered research into all major groups of tumour viruses. Outstanding were the descriptions of the use of restriction enzymes as tools for analysing the genomes of DNA viruses, and the use of pieces of DNA as infective agents in order to define, for instance, the size of the transforming 'gene'.

The small DNA papova viruses have, not surprisingly, yielded most rapidly to analysis with restriction endonucleases. Linear SV40 DNA produced by *Escherichia coli* R₁ endonuclease can transform rat embryo kidney cells (P. J. Abrahams, State University, Leiden), although it can no longer cause productive infection in BSC-1 monkey kidney cells. Transforming activity of restriction fragments smaller than the whole genome is being investigated. A new SV40-specific antigen reported by C. de Vaux St Cyr (Institute of Cancer Research Villejuif) will provide food for thought for genome analysers, already overburdened with gene products to fit into too little virus DNA. This antigen—named 'C'—is cytoplasmic and distinct from T antigen in several other respects. Those who think that polyoma might behave like SV40 will be increasingly disillusioned by reports like that of R. Kamen (Imperial Cancer Research Fund, London), who has found that late in polyoma infection, the major virus-specific RNA is a total transcript of the minus strand—unlike the situation in SV40-infected cells. A transcription map of the polyoma genome is now available, derived from analysis of eight fragments formed by *Haemophilus parainfluenzae* endonuclease II (Hpa_{II}). The origin of replication has been localised in fragment 5 by M. Fried and L. Crawford (ICRF, London). There is a small region of homology with SV40 DNA.

Larger DNA viruses are more complex substrates for restriction analysis, but already considerable progress with adenoviruses (Ad) 2 and 5 has been made. Using a combination of Eco_{RI} and Hpa_I restriction enzymes, P. H. Gallimore (Medical School, Birmingham) has analysed the parts of the adenovirus genome which are present

in Ad2-transformed cells. In some lines as little as 14% of the genome remains. J. F. Williams (Institute of Virology, Glasgow) also reported that two Ad5-transformed cell lines have, respectively, 2½ and 5½ copies of the left end, representing about 30% and 40% of the genome. These findings relate to the question of how much adenovirus DNA is essential for transformation. F. L. Graham (State University, Leiden) has treated rat embryo cells with sheared adeno-DNA. Ad5-DNA will transform these cells even when only about 1×10^6 daltons in size. If half-molecules of Ad5-DNA are made, the transforming activity can be localised in the GC-rich half, and after restriction with Eco_{RI}, it is further localised in the large A fragment, which includes the left end of the molecule. Considering that transformed cell lines exist which only contain the terminal 12% of the left half of the genome, transforming activity must be confined to this site.

Although the problems presented by herpes and Epstein-Barr viruses seem much more immense, these viruses are slowly yielding to the same methods as have been used successfully with small DNA viruses, and their role in human carcinomas is becoming clearer at the same time. Herpes virus (HSV) is a potential candidate for a human tumour-inducing virus. Patients with different carcinomas have been shown to carry HSV-specific non-virion antigens in their sera, and many studies of the transforming potential of HSV or HSV-DNA are now in progress. For example, infection of human embryonic lung cells with HSV is abortive in certain conditions, and cells can become stably transformed (K. Munk, Institute of Virology, Heidelberg). HSV cannot be rescued by cocultivation, so that this may be a good experimental system for studying latency of HSV in human tissue. Another system has been developed by J. C. M. Macnab and N. M. Wilkie (Institute of Virology, Glasgow), who have obtained focal transformation of rat embryo cells by HSV types 1 and 2, as well as by temperature-sensitive (*ts*) mutants and sheared DNA derived either from wild type or from *ts* mutant viruses. As with adenovirus and SV40 DNAs, infectivity of DNA is far more sensitive to shearing than is transforming activity. Sheared HSV-DNA of $5-7 \times 10^6$ daltons (whole genome is 100×10^6 daltons) will induce transformation. J. K. McDougall (Medical School, Birmingham) demonstrated by *in situ* hybridisation that among a population of human cervical carcinoma cells, only very few contain detectable amounts of HSV-DNA, which may explain why whole virus cannot be isolated from the carcinoma. *In situ* hybridisation, be-

cause it allows detection of viral genomes at a cellular level, provides another dimension in the overall analysis of the biochemistry of tumours.

H. Diggelman (Swiss Institute of Cancer Research, Lausanne), introducing a series of papers on RNA viruses, said that a central problem in understanding the function of RNA tumour viruses is still that of determining whether the 70S RNA genome is polyploid. 70S RNA is not found attached to polysomes, nor even in the first budding virus particles, but is apparently assembled later. J. T. Parsons (University of Zurich) has evidence from fingerprint analysis that the genome size is about 3×10^6 daltons—the size of one subunit. Conclusive proof that avian leukaemic myeloblasts contain at least one complete integrated avian myeloblastosis virus (AMV) genome was provided by H. Ogura (Robert Koch Institute, Berlin) and F. Lacour (Institute Gustave Roussy, Villejuif) who have found that AMV production can be induced after infection of helper factor-negative chick cells with leukaemic myeloblast DNA. Similar results were got with wild type or *ts* mutant Rous sarcoma (RSV)-infected cell DNA. The minimum infective size suggests that the information from one RSV subunit may be sufficient. Viral mutants are necessary additional tools in these experiments. R. R. Friis (Robert Koch Institute, Berlin) has isolated representative mutants of the whole replicative cycle in RSV, including one which does not assemble reverse transcriptase into the virion.

Another aspect of RSV transformation was discussed by A. Vaheri (University of Helsinki), who has found that a type-specific glycoprotein, which is normally an integral part of the chick fibroblast cell membrane, disappears on transformation of the cells by RSV. If a *ts* virus mutant is used, the glycoprotein reappears at the non-permissive temperature. W. F. H. Jarrett (University of Glasgow) has studied the horizontal transmission of feline leukaemia virus (FeLV), and the incidence of leukaemia itself as compared with FeLV antibodies in different cat populations. He concludes that one of the primary events in leukaemia may be immunodepression of the response to FeLV antigens themselves. Significantly, the existence of horizontal transmission may allow the development of anti-FeLV vaccines (see *Nature*, **248**, 230; 1974).

Certainly this meeting served to demonstrate again the increasing finesse of the techniques being used to study tumour viruses. One hopes that probing into the mechanism of transformation in these ways will lead to an understanding of the spontaneous events which produce tumours.

Reversible restoration of the birefringence of cold-treated, isolated mitotic apparatus of surf clam eggs with chick brain tubulin

Lionel I. Rebhun

Department of Biology, University of Virginia, Charlottesville, Virginia 22901

Joel Rosenbaum

Department of Biology, Yale University, New Haven, Connecticut 06520

Paul Lefebvre

The Rockefeller University, New York, New York 10021

George Smith

Department of Biology, Union College, Schenectady, New York 12308

Mitotic apparatuses have been isolated which can incorporate heterologous tubulin and can assemble this tubulin into birefringent fibres similar to those of mitotic apparatuses of living cells.

SEVERAL mechanisms have been proposed to explain the separation of chromosomes by the mitotic apparatus (MA). Suggestions such as generation of a tangential force by the spindle body¹, sliding of microtubules^{2,3}, reversible polymerisation of spindle fibres⁴ or participation of a non-microtubule 'movement factor'⁵, possibly actin⁶, have arisen from observations on living or fixed intact cells. Such studies suffer from the inability to experiment directly with the MA except as it exists in the complex whole cell. If it were possible to isolate MAs which could separate chromosomes *in vitro* it should be possible to study those processes relevant to the mechanism of chromosome separation and those that are incidental to it. We report here observations on isolated MAs which (a) incorporate added heterologous tubulin and (b) after removal of homologous tubulin, assemble added heterologous tubulin into birefringent fibres similar in distribution to those of MAs in living cells.

Mitotic apparatus isolation

MAs isolated by methods using organic solvents^{7,8} differ from MAs in living cells in that their birefringence does not disappear at low temperatures. Recent observations by Weisenberg⁹, which demonstrate that calcium ion concentration must be reduced to enable microtubules to be polymerised *in vitro*, suggested that low calcium media be tried in MA isolation procedures. We found that a modification of the microtubule polymerisation mixture described by Weisenberg allows MAs to be isolated from at least eight species of eggs and that the MAs are cold labile, calcium sensitive, stable at room temperature and possess a birefringence approximately equal to that of *in vivo* MAs¹⁰. The isolation mixture consists of 0.1 M MES⁹ or PIPES¹¹ buffer, pH 6.85, 0.25–1 mM MgCl₂, 1–5 mM EGTA, 10 mM of a proteolytic enzyme inhibitor, *p*-tosyl arginine methylester HCl (TAME) and Triton X-100 (0.2–1% depending on the species of egg used). Addition of GTP (1 mM), which is essential for the *in vitro* polymerisation of microtubules^{9,11}, is not required for MA isolation but was included in all reassembly experiments described below. TAME could be eliminated from the isolation mixture but MAs were then obtained in poor yield and were fragmented and irregular in shape. We report experiments using MAs from eggs of the surf clam *Spisula solidissima*.

Eggs of the clam were obtained and handled as previously described^{12,13} and were parthenogenetically activated with KCl. Fully formed first meiotic MAs at metaphase were uniformly present by 12–14 min after activation¹². Vitelline membranes were removed with 1 M glycerol solutions¹⁴ and the eggs were immediately suspended in 2–3 times their volume of isolation medium with Triton X-100 at room temperature. The eggs were then dispersed by agitation with a Vortex mixer and the MAs collected by centrifugation at 400–500*g* for 1.5–2 min at room temperature. The MAs were then resuspended in at least five times their volume of isolation medium without Triton X-100 (Fig. 1*a*). Measurements of the birefringence of the isolated MAs were made with a Zeiss polarisation microscope (Photomicroscope II) equipped with a Brace-Koehler compensator and a potentiometric device for automatic recording of compensator angle.

Chick brain tubulin was routinely isolated by homogenising the brains of 1–3-d-old chicks in a volume of polymerising solution (1 mM EGTA, 1 mM GTP, 1–2 mM, MgCl₂, 0.1 M PIPES buffer, pH 6.9) equal to 1.5 times the wet weight of the brains. The homogenate was centrifuged for 1 h at 28,000*g* at 4° C. The supernatant was then incubated at 37° C for 45 min until it became very viscous as a result of the forma-

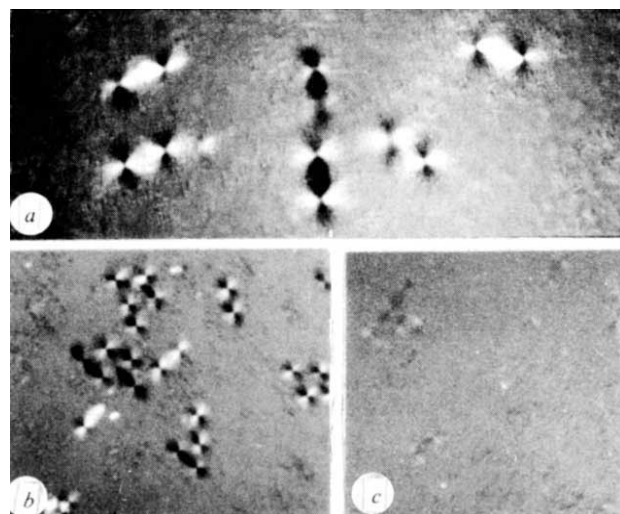


FIG. 1 *a*, Isolated MAs of *Spisula* eggs at metaphase in isolation medium. Distance between astral centres averages 22 μ m. $\times 2,500$. *b*, Isolated MAs in isolation medium to be compared with the same preparation cooled to 4° C for 20 min. *c*, Considerable decrease in birefringence is visible and on measurement amounts to one-quarter to one-half the birefringence originally present. $\times 1,000$.

TABLE 1 *Spisula* mitotic apparatus isolated at metaphase at 22–24° C

Treatment of MAs		Retardation*	% of control retardation
Control	Normal MAs in isolation mix at 25° C	2.70 μ m	100%
	Normal MAs in isolation mix at 4° C for 15 min	1.25 μ m	46%
Tubulin	MAs in tubulin (9 mg ml ⁻¹) at 25° C for 15 min	3.70 μ m	137%
	MAs in tubulin (9 mg ml ⁻¹) at 4° C for 20 min	1.10 μ m	41%
	MAs from above, removed from cold and incubated at 30° C for 15 min	2.40 μ m	90%

* Each retardation is based on 10–13 measurements. The tubulin was purified from chick brain

tion of microtubules. The microtubules were collected from the supernatant by centrifugation at 28,000*g* for 15 min at 25° C. The sediment of microtubules was resuspended in an amount of polymerising solution equal to one-third to one-quarter the volume of the original supernatant and placed on ice for 30 min to depolymerise the microtubules. This preparation was centrifuged at 105,000*g* for 1 h to remove any remaining intact microtubules. The supernatant which contained the tubulin subunits was either used immediately for polymerisation with the isolated MAs or, in some experiments, was further purified by one or two additional cycles of polymerisation and depolymerisation. Tubulin from chick brain was used rather than tubulin from *Spisula* eggs because it was much easier to obtain in large amounts, and other work had recently shown that heterologous systems of chick brain tubulin and sea urchin flagellar axonemes could copolymerise¹⁵.

Birefringence

The rapidity with which cold treatment reduced the birefringence of isolated MAs was strongly dependent on the time between MA isolation and immersion in a cold bath.

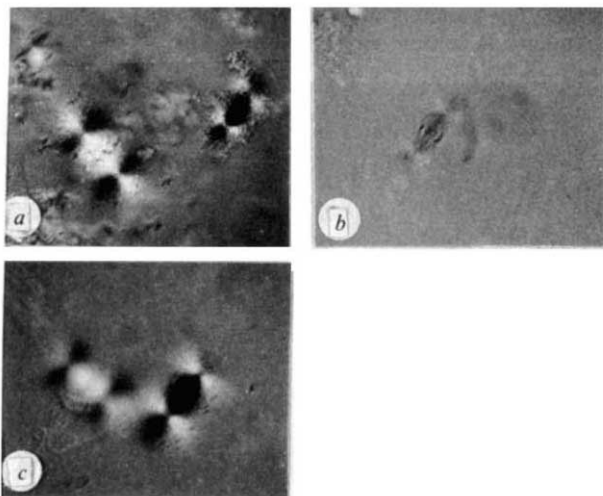


FIG. 2 *a*, MAs were isolated and incubated in concentrated tubulin solutions at room temperature for 30 min. They were then centrifuged out of tubulin, washed in isolation medium and mixed with intact MAs from the original isolation which had remained in isolation medium at room temperature. The difference in size is readily apparent and is uniform. $\times 2,500$. *b*, The MA remnant results from cooling in tubulin and should be compared with the original MAs in tubulin at room temperature. *c* was taken several minutes after suspension of intact MAs in tubulin and significant growth had already occurred. $\times 2,500$.

MAs cooled to 0–4° C within 1 min of isolation completely disappeared and were not visible in the polarisation microscope. Those treated 5–6 min after isolation required only 10–15 min at low temperature to reduce their birefringence to a constant level about one-quarter to one-half that of normal, (Table 1 and Fig. 1*b* and *c*) whereas if greater than 15 min elapsed between isolation and cooling, reduction of birefringence was delayed for several hours. In the latter cases a remnant survived which had the form of an MA with considerably reduced birefringence¹⁰. Electron microscopic observations showed that microtubules were absent from cold-treated MAs with reduced birefringence but that such MAs contained chains of ribosome-like particles aggregated with some amorphous material¹⁰: it is this material which presumably gives rise to the residual form birefringence of the MA remnant^{16,17} (Fig. 1*c*). The MAs depleted of tubules still contained the chromosomes and the mitotic poles (centrosomes).

Tubulin assembly

Intact MAs to be resuspended in brain tubulin were obtained in isolation medium, centrifuged once at low speed and then resuspended in IM plus tubulin at the desired concentration. This procedure, which diluted the Triton X-100 concentration to less than 0.1% and reduced the concentration of free clam tubulin subunits, took 5–6 min. Such suspensions of intact MAs, in solutions containing up to 10 mg ml⁻¹ chick brain tubulin (approximately the concentration of tubulin in intact eggs^{18,19}), in contrast to MAs suspended in isolation medium alone, retained their cold lability for at least 1 h. If these intact MAs were kept at room temperature in tubulin they increased their birefringence over 15 min (Table 1) with little observable change in shape. At greater concentrations of tubulin (15–20 mg ml⁻¹) the MAs became considerably wider at the equator, the length of the astral fibres increased and the distance between astral centres increased by up to 40% (Fig. 2*a*). These results show that chick brain tubulin can assemble into clam MAs which still contain clam microtubules.

Perhaps the most interesting observations in these studies concern the return of birefringence in MAs which were depleted of their own microtubules by cold treatment and were then warmed to 30° C in the presence of chick brain tubulin. In these experiments, the isolated MAs were sedimented from isolation medium and then resuspended in isolation medium + tubulin (9 mg ml⁻¹). Control MAs were suspended either in isolation medium alone or in isolation medium with tubulin and 10⁻³ M colchicine. The MAs were then cooled to 4° C for 15 min and the birefringence was reduced by about 60% (Fig. 2*b* and *c* and Table 1). When cold-treated MAs were incubated at 30° C the birefringence in the preparations which contained isolation medium and tubulin partially returned in 15 min, (Table 1) and within 0.5–0.75 h the birefringence had returned to the level seen before cold treatment. Moreover, these MAs continued to increase in size and after 2 h at 30° C many were three to four times as long as the normal MAs (Fig. 3*a* to *d*). Control MAs in the presence of isolation medium alone or which contained colchicine in addition to the tubulin showed no restoration of normal birefringence when warmed from 4° C to 30° C. Reassembled MAs could be cold depolymerised after periods of up to 2 h and on addition of fresh GTP and rewarming to 30° C birefringence returned to the cold-treated remnant although such doubly-restored MAs were generally less birefringent than those restored once. The doubly restored MAs were still cold labile. Restored MAs could be spun out of tubulin solutions and suspended in isolation medium alone, in which they were stable at room temperature. Thus, restored MAs, resembling those isolated directly in isolation medium, are stable to dilution of the tubulin subunits in which they were for-

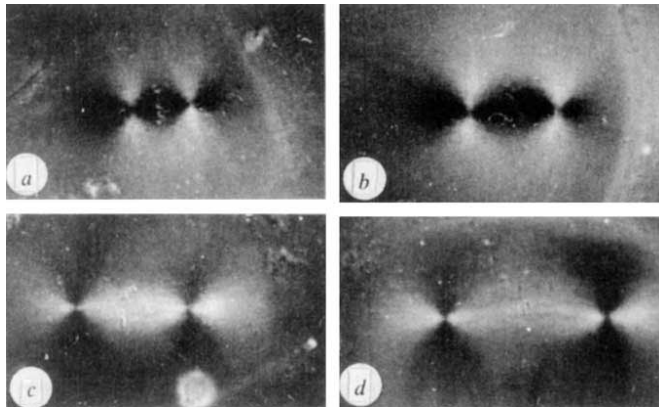


FIG. 3 *a*, Example of a MA depleted of tubules incubated in tubulin at 30° C. for 30 min. Compare with the MAs in Fig. 2*b* and *c*. Note the metaphase chromosomes. $\times 2,500$. *b-d*, MAs from the same preparation as in (*a*) but incubated for 1 h (*b*) or 1.5 h (*c* and *d*). The length of the MA in *d* is approximately 3.5 times normal (compare Fig. 2*c*). At least half of the reconstructed MAs in the preparation attained lengths about that of the MA in *c*. $\times 2,500$.

merly bathed. The birefringence of restored MAs is removed by 10^{-3} M calcium but is stable in 10^{-5} M calcium. This range of calcium concentration would be expected from *in vitro* observations on neurotubule polymerisation where microtubule assembly can take place and the microtubules remain stable in 10^{-4} M but disappear at 10^{-3} M calcium. These results show that brain tubulin can reversibly assemble into clam MAs which have been depleted of their own microtubules by cold and that such reassembled MAs are cold labile, calcium sensitive, stable to dilution and capable of considerable increase in overall length.

Attempts were made to follow the reformation of birefringent fibres in the MA remnants. Birefringence appeared to return throughout the MA rather than at any focal point. Similarly, during cold treatment, birefringence is lost all along the MA which does not shorten in length. Since *in vivo* MAs decrease in length as well as birefringence on being slowly cooled to low temperature⁴ the question of the behaviour of the isolated MAs compared with those *in situ* may be raised. *In vivo* *Lytechinus* MAs at first cleavage, however, lose birefringence simultaneously along the whole MA when cooled rapidly with large temperature jumps and restore birefringence uniformly along the MA when the eggs are returned to the original temperature²⁰. This behaviour more nearly resembles our results of restoration of birefringence in cold-depleted MAs.

It is important to note that MAs isolated by our techniques increase in length but do not shorten and that they are

stable to dilution of tubulin. This indicates that they can incorporate tubulin but that they can not disassemble it easily once it is integrated. Since disassembly as well as assembly is an important part of the process of chromosome separation⁴ and it did not seem to occur in the metaphase MAs, we attempted to isolate MAs at anaphase. This proved difficult because of the very short anaphase time in *Spisula* and because such MAs were considerably more fragile than metaphase MAs and tended to fragment. Nevertheless, some anaphase MAs were obtained (Fig. 4*a*) whose birefringence was cold labile and could be restored when incubated in tubulin solutions. In some cases, cold depleted anaphase MAs incubated in tubulin for about 1 h attained a configuration resembling very late anaphase or early telophase, (Fig. 4*b*) with chromosomes at the centres of quite small asters. Details of how this configuration was achieved are now being investigated.

We have described a technique for isolating MAs which can incorporate heterologous tubulin into the existing intact MA and which, after depletion of native microtubules by cold treatment, can utilize added tubulin to reconstruct the birefringent material^{15,16,18,21} in approximately normal distribution. Such MAs continue to grow in tubulin solutions at room temperature or above and can grow considerably longer than normal. The ability to incorporate exogenous tubulin is at least one of the functions of the *in vivo* MA and is part of the process of chromosome separation. Whether our results with anaphase MAs are due to disassembly of microtubules from the isolated MAs is not clear but it may now be possible with proper modification of the medium and variation of tubulin concentration to obtain chromosome separation *in vitro* in mass-isolated MAs.

Similar experiments have been carried out recently in other laboratories in which observations have been made on MAs of single cells perfused with tubulin solutions^{22,23}.

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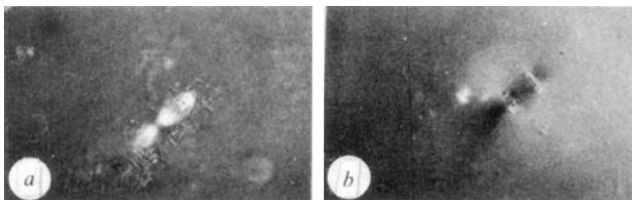


FIG. 4 *a*, MA at anaphase in isolation medium. $\times 2,500$. *b*, Regrown MA from an anaphase preparation after cold treatment. Chromosomes appear to cluster about the astral centres. Although numerous anaphases were present in the original preparation (as well as metaphase MAs) after about 1 h at 30° C only metaphase and 'telophase' configurations could be seen. The birefringence of the region between chromosome clusters in the latter is lower than that in chromosome-pole regions of metaphase MAs in the same preparations. $\times 2,500$.

Roles for *E. coli* DNA polymerases I, II, and III in DNA replication

Robert C. Tait* & Douglas W. Smith

Department of Biology, University of California, San Diego, La Jolla, California 92037

Chain elongation during E. coli DNA replication proceeds in three distinct stages. Participatory roles of the three E. coli DNA polymerases in each of these stages has been determined.

ALTHOUGH the overall process of DNA chain elongation during DNA replication is semiconservative¹, the biochemical events involved are unknown². *E. coli* DNA polymerase I (PolI) cannot catalyse *in vitro* DNA synthesis in a semi-conservative manner using isolated DNA as a template³. *E. coli* *polA* mutants⁴, deficient in PolI, grow normally, suggesting that PolI is not an essential enzyme. *PolA* mutants have been used to purify further DNA polymerising enzymes including DNA polymerase II (PolII) and DNA polymerase III (PolIII)⁵⁻⁸. *E. coli* *polB* mutants^{9,10} deficient in PolII, and *dnaE* mutants^{11,12}, deficient in PolIII, have been isolated and studied. *PolB* mutants are similar phenotypically to their *polB*⁺ parental strains, although differences in DNA repair ability have been detected^{13,14}. In contrast, only thermosensitive *dnaE* mutants have been isolated, suggesting that PolIII is an essential enzyme.

PolI preferentially catalyses a 'repair' polymerisation reaction *in vitro*: conversion of partially single-stranded DNA (ssDNA) into the double-helical species. Although optimal reaction conditions are different, the polymerisation reactions catalysed by purified PolII and PolIII *in vitro* are also substantially 'repair' reactions^{7,15}.

In the work reported here, the physiological roles of the three known *E. coli* DNA polymerases in DNA replication were examined. The temporal fate of newly replicated DNA obtained from *E. coli* mutants deficient in one or more of these DNA polymerases was analysed using alkaline sucrose gradients. Results indicate that PolIII is essential for one elongation step and involved, if not essential, in a second, that PolI is involved but not essential for a third elongation step, and that PolII is needed and involved only in the absence of one or both of the other two enzymes (Fig. 4).

Pol I

SsDNA, labelled for 1 min in W3110 *polA*⁺ cells, sedimented in a broad band when synthesised at both 30° C (Fig. 1a) and 43° C (Fig. 1c). The sedimentation values (30S to 110S) corresponded with molecular weights intermediate to parental sized 120S to 130S ssDNA and the short newly synthesised 15S to 20S ssDNA fragments¹⁶. In contrast, 1-min-labelled ssDNA from p3478 *polA1* cells⁴, deficient in polI (0.5%–2% W3110 activity¹⁹), sedimented more slowly (Fig. 1b and d) when synthesised either at 30° C (10S to 40S) or at 43° C (15S to 50S). During subsequent DNA synthesis in both strains (10 min and 40 min cold chase profiles), the short DNA pieces were converted first into intermediate sized pieces and then into large pieces, at a rate somewhat slower at 30° C than at 43° C. The rate of conversion was slower in p3478 than in W3110, both at

30° C and at 43° C, in agreement with earlier observations^{17,18}. Thus, PolI is required for rapid conversion of newly synthesised DNA into intermediate sized pieces. The residual slow rate observed in p3478 may be due to residual PolI activity¹⁹ or to attempts by another DNA polymerase to substitute for PolI.

Pol III

E. coli E486 *dnaE*486 is a temperature-sensitive *E. coli* mutant¹¹ with normal PolI and PolII activities and no detectable PolIII activity *in vitro* at either 30° C or 43° C²⁰. The sedimentation profiles (Fig. 2a) of ssDNA synthesised by E486 at 30° C are similar to those of ssDNA from W3110 cells (Fig. 1a). The increased relative amount of slowly sedimenting DNA synthesised for 1 min in E486 suggests that PolIII is partially defective *in vivo* in E486 at 30° C, as *in vitro*²⁰.

The sedimentation profiles of DNA synthesised at 43° C by E486, however, are strikingly different (Fig. 2b). DNA synthesis in E486 was greatly reduced at 43° C and occurred mainly within the first minute after shift to 43° C. Most of the DNA synthesised for 1 min or 3 min sedimented slowly (20S or less). During the subsequent 7 min and 37 min cold chase, about half of the labelled DNA was converted slowly into intermediate sized pieces, with some DNA degradation. Even after 40 min of synthesis at 43° C, however, about half of the initially synthesised DNA continued to sediment slowly. Much slowly sedimenting ssDNA remained even after 80 min synthesis at 43° C (not shown). Thus, PolIII is required for the complete conversion of short DNA pieces into intermediate sized pieces, and, when PolIII is defective, the rate of synthesis, or processing, of these short pieces is greatly reduced. The slow rate of the observed partial conversion may have been due to residual *in vivo* PolIII activity at 43° C or to an attempt by another DNA polymerase to catalyse this synthesis. Further, nearly all the ssDNA sedimenting more rapidly than about 20S was found as intermediate sized 70S to 120S pieces, even after 80 min of synthesised and/or processed, they were converted rapidly into intermediate sized pieces.

Very little DNA was found at any time in the region 30S to 70S, suggesting that once the 20S short pieces were fully synthesised and/or processed, they were converted rapidly into 70S to 120S intermediate sized pieces. Figure 1 shows that PolI, when present, was responsible for the rapid conversion. This role of PolI in the synthesis of the intermediate sized pieces was further examined using *E. coli* E4862, a *polA1* derivative²⁰ of E486. Sedimentation profiles of DNA synthesised by E4862 at either 30° C or at 43° C (Fig. 2c) are identical to those from E486, except that a substantial fraction of the 10 min and 40 min ssDNA from E4862 sedimented as 30S–70S material. Thus, when PolI activity was greatly reduced, processed short DNA pieces were converted slowly into 70S to 120S pieces, whereas in the presence of wild-type PolI activity, this conversion occurred sufficiently rapidly that very little 30S to 70S DNA was observed.

The slow conversion of short DNA pieces into intermediate

* Present address: Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, California 94122

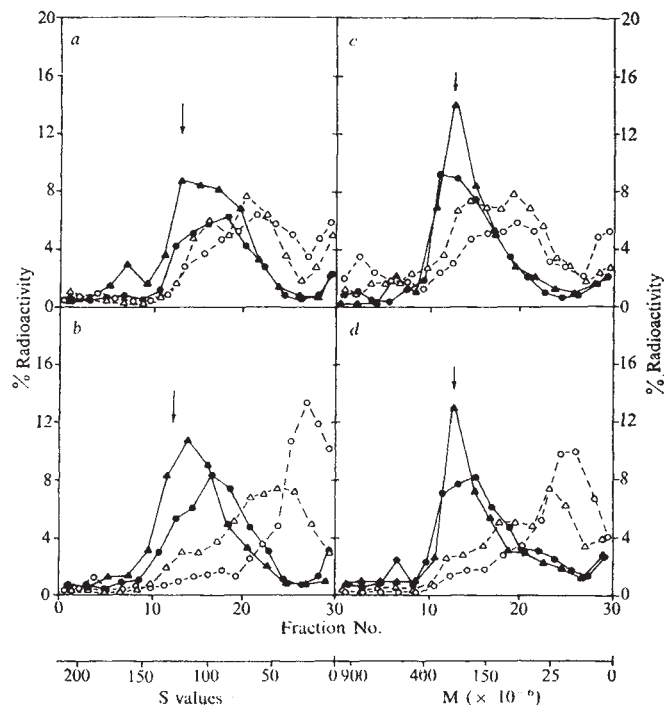


Fig. 1 Alkaline sucrose gradients of DNA from W3110 and p3478. *E. coli* W3110 *thy*⁻ and p3478 *thy*⁻ *polA1* were grown in a Tris-glucose-minimal salts (TG₀) medium containing 0.1% casamino acids and ¹⁴C-thymine (2 μ Ci ml⁻¹, 2 μ g ml⁻¹) at 30° C to 2×10^8 cells ml⁻¹. Half of each culture was collected by centrifugation (4° C, 5 min, 10,000 *g*), resuspended in an equal volume of TG₀ minus glucose, and added to a supplement containing ³H-thymidine, to give 6 μ Ci ml⁻¹, and glucose, casamino acids and thymine in amounts to yield TG₀ plus 0.1% casamino acids. The cultures were incubated at 30° C with aeration. After 1 min and 3 min, quarter volume samples were frozen immediately in dry ice-acetone. At 3 min, non-radioactive thymidine to 50 μ g ml⁻¹ was added. At 10 min and 40 min, two further quarter volume samples were frozen immediately. The remaining halves of each of the two original cultures were shifted to 43° C for 15 min, collected by centrifugation (4° C, 5 min, 10,000 *g*), resuspended in prewarmed TG₀ minus glucose, added to supplement, and incubated at 43° C with aeration. Samples were frozen at the four time points, and the cultures were chased with non-radioactive thymidine, as described above. 43° C was attained within 30 s after cultures were placed at 43° C. A 1 ml sample of each frozen cell suspension was thawed, the cells were converted into lysozyme-EDTA spheroplasts, and $\leq 5 \times 10^7$ cells (50 μ l of each sample) were lysed by layering gently into 0.1 ml 0.5 N NaOH over a 4.7 ml alkaline sucrose gradient (5%-20% linear gradient in 0.1 N NaOH), using the procedures of Smith and Meun³⁵. Following centrifugation (30,000 r.p.m., 15° C, 105 min, Spinco SW50.1 rotor), fractions were collected directly onto Whatman 3 MM paper squares. These were dried, washed twice in cold 5% Cl₂ CCOOH, twice in 95% ethanol, once in acetone, dried, and assayed for radioactivity. The total radioactivity layered on each gradient was 7,000-35,000 c.p.m. for ³H and 500-1000 c.p.m. for ¹⁴C. Sedimentation coefficients and molecular weights were determined using known relations^{36,37} and phage T4 DNA as a standard. ¹⁴C-DNA sedimentation profiles were similar in each gradient and nearly identical to the 40 min ³H-DNA profiles; the peak of the ¹⁴C-DNA is indicated by the arrow. a, W3110 *polA*⁺, 30° C; b, p3478 *polA1*, 30° C; c, W3110 *polA*⁺, 30° C; d, p3478 *polA1*, 43° C. ○, 1 min ³H-DNA; △, 3 min ³H-DNA; ●, 10 min ³H-DNA; ▲, 40 min ³H-DNA.

sized pieces observed in E4862 at 43° C (Fig. 2c) may involve participation of PolII, as E4862 is deficient in PolI and PolIII at 43° C, although residual PolI and/or PolIII activity *in vivo* at 43° C is possible. We examined these possibilities further using an independent *dnaE* mutant, *E. coli* BT1026 *polA1 dnaE1026*. BT1026 possesses greatly reduced PolII activity, normal PolIII activity, little detectable PolIII activity (<1% wild type) at 42° C, and reduced

PolIII activity (<10% wild type) at 30° C *in vitro*^{10,20,21}. Although sedimentation profiles of DNA synthesised by

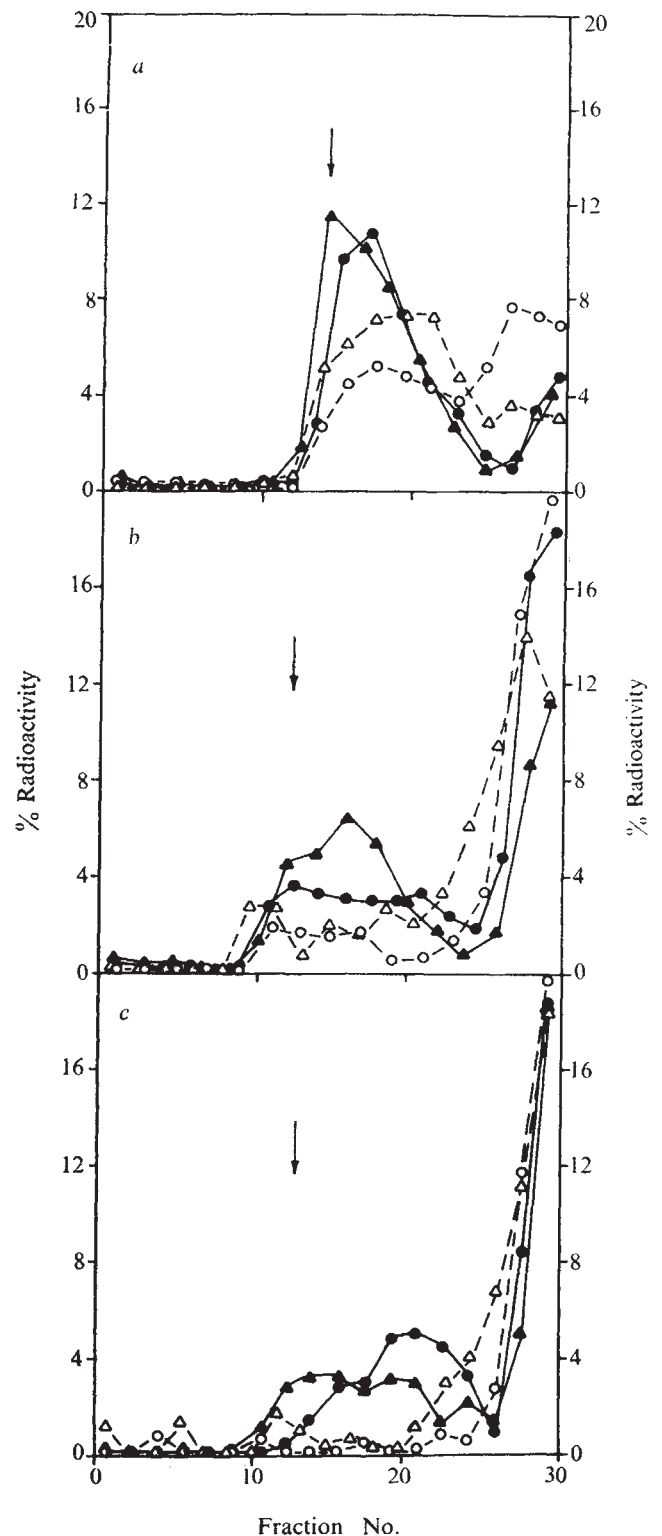


Fig. 2 Alkaline sucrose gradients of DNA from E486 and E4862. *E. coli* E486 *thr*⁻ *leu*⁻ *thy*⁻ *lac*⁻ *met*⁻ *dnaE486* and E4862 *arg*⁻ *his*⁻ *thy*⁻ *polA1 dnaE486* were separately cultured and the DNA was labelled and analysed as described in the legend to Fig. 1. E4862 *thy*⁻ was obtained from E4862 *thy*⁺ using trimethoprim selection³⁸. The types of curves are as in Fig. 1; the ¹⁴C DNA profiles were similar to the 40 min ³H-DNA profile in (a) in each case, with the peak indicated by the arrow. The total radioactivity was 900-13,000 c.p.m. for ³H, and 1,000-5,000 c.p.m. for ¹⁴C. a, E486 *polA*⁺ *dnaE486*, 30° C; b, E486 *polA*⁺ *dnaE486*, 43° C; c, E4862 *polA1 dnaE486*, 43° C.

BT1026 at 30° C (Fig. 3a) are similar to those of p3478 (Fig. 1b) as expected, much of the ssDNA from BT1026 sedimented slowly even after the 37 min chase. These features suggest that PolIII in BT1026 is also partially defective at 30° C *in vivo*, as *in vitro*²⁰, but to an even greater extent than in E4862. The above sedimentation properties of the DNA synthesised by BT1026 at 43° C were considerably accentuated (Fig. 3b). The amount of DNA synthesised was reduced greatly, and some degradation occurred in time. Most of the DNA sedimented slowly even after a 37 min chase and the larger pieces sedimented as intermediate sized pieces. Since these sedimentation properties are similar to those of ssDNA from the independent *dnaE* mutant E4862 (Fig. 2c), residual PolIII activity at 43° C probably was not involved in the slow conversion of short DNA pieces into intermediate sized pieces.

A less complete conversion by BT1026 of short DNA pieces into intermediate sized pieces occurred at 43° C than at 30° C (compare Fig. 3a and b), suggesting that PolIII, when PolII activity is reduced, participates in this conversion process. This possible role for PolIII is obscured, however, by the requirement for PolIII in the maturation of the short DNA pieces.

Pol II

A role for PolII in this slow conversion was examined using a mutant of BT1026 deficient in PolII. This mutant, H10265, has no detectable PolII activity at 43° C and very little (<0.02% wild type) PolII activity at 30° C²⁰. The amount of DNA synthesised by H10265 is similar to that by BT1026 at 30° C. However, two differences in the ssDNA sedimentation profiles are apparent. First, the rate at which short ssDNA pieces were converted into intermediate sized pieces was reduced in H10265 (3 min DNA distributions, Fig. 3a and c). This suggests that, when PolI is deficient, PolII participates in the conversion of short 20S ssDNA fragments into larger 70S to 120S pieces. Second, the 40 min DNA from H10265 sedimented as rapidly as did the parental DNA. Since PolIII seemed to be partially defective *in vivo* in BT1026 at 30° C (Fig. 3a), the presence of PolII, when PolIII is partially defective, apparently prevents conversion of 70-120S DNA into the size and/or shape typical of parental ssDNA. Very little DNA synthesis was observed in H10265 at 43° C; the resulting sedimentation profiles were similar to those of BT1026 at 43° C (Fig. 3b).

Further evidence supporting these proposed roles for PolII in DNA replication was obtained in similar studies using *E. coli* HMS83, PolB derivative of p3478 containing no detectable PolII activity *in vitro*⁹. First, the rate of conversion of short ssDNA pieces in HMS83 to intermediate sized pieces was reduced when compared with p3478 (data not shown), again suggesting that PolII, when PolI activity is reduced, participates in this conversion. The slow residual conversion observed in HMS83 was probably due either to residual PolI activity¹⁹ or to PolIII activity. Second, intermediate sized pieces were converted into parental sized ssDNA within 40 min at 30° C, indicating that neither PolII nor PolIII were required for this conversion when PolIII was present at wild type levels.

Three stages in chain elongation

Our results can be summarised in the schematic model shown in Fig. 4. Three stages in DNA chain elongation are visualised. In the first stage, synthesis of a short DNA fragment is initiated, the fragment is elongated to a 20S piece, and processed into a form ready for further elongation. These pieces are the short DNA pieces discovered by Okazaki *et al.*¹⁶ When PolIII is deficient, synthesis of these pieces and/or their maturation proceeds very slowly and incompletely. In the second stage, the 20S pieces are converted into intermediate sized 70S to 120S pieces. This conversion pro-

ceeds very rapidly when PolII is present in the cell compared with the first stage of chain elongation. When PolII is deficient, both PolII and PolIII can participate in this conversion. In

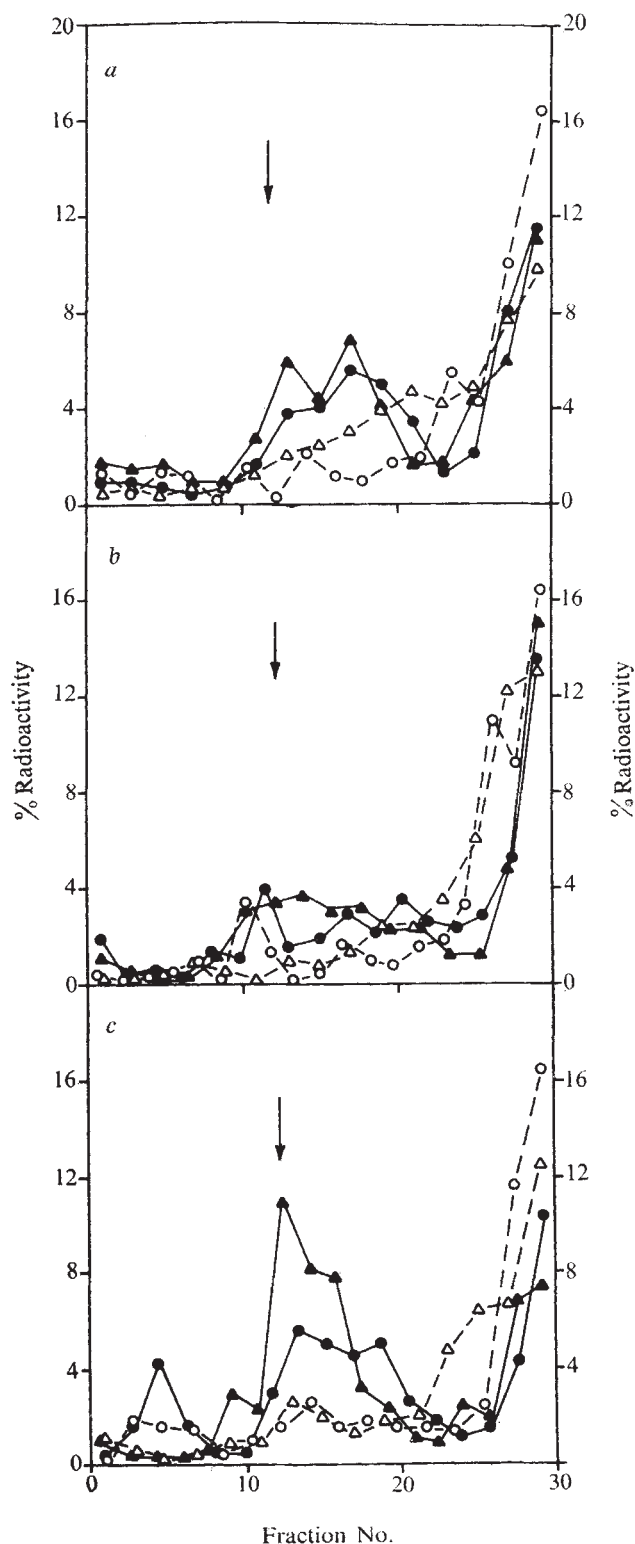


FIG. 3 Alkaline sucrose gradients of DNA from BT1026 and H10265. *E. coli* BT1026 *thy-endA-polA1, dnaE1026* and H10265 *thy-endA-polA1 polB- dnaE1026* were cultured separately and the DNA was labelled and analysed as described in the legend to Fig. 1. The types of curves are as in Fig. 1; the ¹⁴C-DNA profiles were similar to the 40 min ³H-DNA profile in (c) in each case, with the peak indicated by the arrow. The total radioactivity was 500–2,000 c.p.m. for ³H, and 900–5,000 c.p.m. for ¹⁴C. a, BT1026 *polA1 dnaE1026*, 30° C; b, BT1026 *polA1 dnaE1026*, 43° C; c, H10265 *polA1 polB- dnaE1026*, 30° C.

the third stage, intermediate sized pieces are converted into parental sized pieces. In cells containing PolII, PolIII is required for this process. However, when PolII is deficient in a cell containing a defective PolIII, the conversion seems to take place.

Although biochemical reactions could not be determined in our experiments, the following possibilities seem worth mentioning. PolIII is required for elongation and complete synthesis of the most newly synthesised Okazaki DNA pieces. The RNA found covalently attached to these pieces²² may serve as a primer for initiation of synthesis of these short pieces, permitting a subsequent elongation reaction similar to the preferred gap-filling reaction catalysed *in vitro* by PolIII^{15,23}. This elongation could continue until a termination signal were reached, such as running into the adjacent short DNA piece, terminating stage I.

To begin stage II, the products of stage I may need to be processed. Attainment of a fixed size may suffice. Alternatively, removal of the putative RNA primer may be involved. A subsequent rapid repair of the gap left by the excised RNA, coupled with a ligase joining of the individual short pieces, could result in the 70S to 120S pieces. Since this conversion occurs very rapidly in cells containing PolI, PolI may serve as an RNase H²⁴, catalysing the exonucleolytic removal of the RNA from the RNA-DNA duplex. Although *E. coli* contains a distinct RNase H activity²⁵, PolI possesses RNase H activity *in vitro*²⁶ and seems to have an RNase H role *in vivo* during colE1 DNA replication²⁷. When PolI activity is substantially reduced, either PolII or PolIII, or both, may possess RNase H activity and remove the RNA primer. Alternatively, the *E. coli* RNase H may remove the RNA, and PolII and/or PolIII may fill in the gap left by the excised ribonucleotides. Joining of these matured short DNA pieces by a ligase would yield the 70S to 120S pieces.

In stage III, the intermediate sized pieces (50–250 × 10⁶ daltons) are converted into parental sized pieces. For a chromosome of 2,500 × 10⁶ daltons, 5 to 25 such pieces would exist per chromosome. As an appealing hypothesis, the products of stage II may correspond to the newly synthesized DNA strands of the 12 to 80 loops found in the *E. coli* folded chromosome²⁸ by Worcel and Burgi²⁹. Stage III would then comprise the biochemical events joining these loops together to yield the supercoiled folded bacterial chromosome²⁹. This hypothesis also provides a rationale for involvement of PolIII in stages I and III. Considerable indirect evidence for a replication site on the bacterial cell surface has accumulated^{30–34}, and PolIII seems to prefer a hydrophobic environment^{15,21}. Worcel and Burgi²⁹ propose that the 12 to 80 loops may emanate from an RNA core, since RNA is essential for the integrity of the folded chromosome²⁸. Such an RNA core could be part of the DNA replication site at the cell surface. Stage I could then take place in the hydrophobic environment of the DNA replication site, with PolIII as the primary DNA replicase, whereas stage II might not require this environment and/or site and could occur in the cytoplasm. Stage III could then again occur at the repli-

cation site, as the Worcel-Burgi loops projecting into the cytoplasm from the replication site were joined together. This hypothesis would then partially compartmentalise the *E. coli* DNA polymerases, localising PolIII to the hydrophobic environment of the cell surface, thus in part explaining the need for different DNA polymerase enzymes to catalyse similar gap-filling polymerisation reactions.

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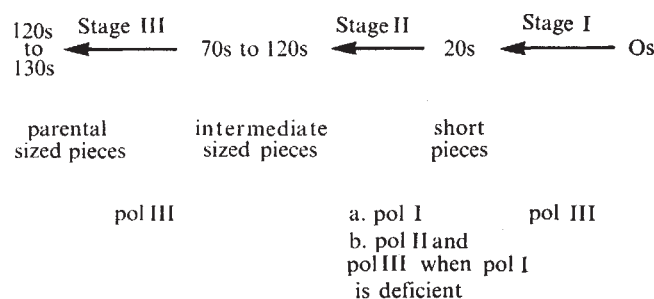


FIG. 4 Three stages of chain elongation during *E. coli* DNA replication.

Barbed bone points from Central Sudan and the age of the "Early Khartoum" tradition

D. Adamson

School of Biological Sciences, Macquarie University, New South Wales 2113, Australia

J. D. Clark

Department of Anthropology, University of California, Berkeley, California 94720

M. A. J. Williams

School of Earth Sciences, Macquarie University, New South Wales 2113, Australia

Barbed bone points, typical of those from the early Holocene settlement of "Early Khartoum", have been found at three sites along the White Nile, south of Khartoum. The form of the fragments and the stratigraphy of the sites throw light on the environment and technology of the early settlements along this part of the Nile.

DURING archaeological and geomorphological fieldwork along the White Nile south of Khartoum in February, 1973 (see Fig. 1), we found at three sites, fragments of barbed bone points typical of those from the early Holocene settlement of "Early Khartoum"¹. No radiocarbon dates exist for the "Early Khartoum" settlement, but at El Qoz² the characteristic pottery is found stratified below that associated with the Neolithic settlement at Esh Shaheinab, the average date of which is $5,253 \pm 415$ yr BP (ref. 2, page 107). One of our discoveries, from near the village of Tagra, is of particular interest because it came from a depth of 1.3 m in a soil pit for which two radiocarbon dates are already available. A second discovery was made on an ancient point bar of the White Nile, in swamps opposite Esh Shawal near Guli. The third site, which we studied in some detail, was located at Shabona and yielded an industry identical to that from "Early Khartoum". Shabona is a settlement site and only the second with this cultural pattern to have been found and excavated. A similar site was located about 3 km distant from Shabona so that it seems likely that other similar riverside settlements would be found in this part of the White Nile between Khartoum and Kosti.

Tagra site

Williams³ in 1963 collected subfossil freshwater and amphibious Mollusca from 1.6 to 1.9 m depth in a soil pit near Tagra, the shell giving a radiocarbon date of $8,370 \pm 350$ yr BP (Isotopes Inc. 1485, dated by courtesy of Professor A. J. Whiteman). This pit was resampled by Williams and Adamson in 1972 and a shell radiocarbon date of $8,130 \pm 225$ yr BP (Sydney University Radiocarbon Laboratory SUA-68) was obtained from a depth of between 1.2 to 1.4 m. In February 1973, Adamson extended and sampled this pit yet again for more shell material and found, at a depth of 1.3 m, two fragments of a barbed bone point, fragments of small bones of mammal, fish bones including cat fish spines, and shells of *Pila wernei* (Philippi) and *Lanistes carinatus* (Oliver). The presence of large complete *Pila* up to 9 cm long suggests deposition of the shells and bone *in situ* and lack of disturbance by soil cracking and mixing.

The location and stratigraphy of the Tagra pit are shown in Fig. 1. The pit is located 230 m on a 42° bearing from the steel beacon marking the surveyed position of 13°56'N, 32°22'E and it lies about 0.5 km east of the present 377.8 m high level of the White Nile as controlled by the Jebel Aulia dam. The pit surface is between 378.2 and 378.6 m which is somewhat lower than the elevation first reported⁴. Before the dam was built, local mean low water level was 372.2 m and local mean flood level was 375.4 m at the nearby Ed Dueim gauge⁵. The pit surface is, therefore, about 6 m above the former low level, about 3 m above the former high level and about 0.4 to 0.9 m above the present high water level caused by the Jebel Aulia dam.

The barbed point fragments, other bones and shells just mentioned, occurred near the base of a dark-grey clay 1.5 m thick, which overlies a calcic layer 10 cm thick (1.5 to 1.6 m) containing abundant hard calcium carbonate nodules and softer calcareous aggregates embedded in fine sand and silt. Beneath the calcic layer is 15 cm of olive-grey finely laminated silts (1.6 to 1.75 m) with thin bands of reddish-yellow limonite accentuating the bedding planes. From 1.75 to 1.9 m, very pale greyish-brown clayey sand contains occasional shells of *Pila wernei* and *Melanoides tuberculata* (Müller). From 1.9 to 2.05 m there are alternating bands of rippled brown clay and grey or yellow sand, each band being about 0.5 to 1.0 cm thick. Occasional strongly calcareous lenses occur in this horizon. The shells for the $8,370 \pm 350$ yr BP date came from between 1.6 and 1.9 m and were *in situ*. Light olive-grey clayey sand from 2.05 to 2.20 m formed a transitional layer above light grey fine quartz sand at least 60 cm thick. The total depth examined was 2.85 m.

Sequence of events at Tagra

A tentative interpretation of the sequence of events at this site is offered. Fluvial fine sands and clayey sands were laid down in the White Nile up to at least 376 m, that is about 4 m above the normal minimum water level before the Jebel Aulia dam was built. A further 30 cm of fluvial aggradation occurred and both aquatic and semi-aquatic molluscan shells were incorporated in the silts, clays and sands some 8,000 to 8,500 yr ago at an elevation at least 0.5 to 1.0 m above the pre-dam flood level. The presence of *Melanoides tuberculata* indicates permanent water and *Pila wernei* and *Lanistes carinatus*, being amphibious species, could indicate seasonal inundation. Their presence suggests a mixed assemblage of shells such as could occur at the boundary between permanent water and seasonally inundated swamps. The close alternation of rippled sands and clays in the horizon immediately below laminated grey silts is consistent with seasonal deposition between high and low water

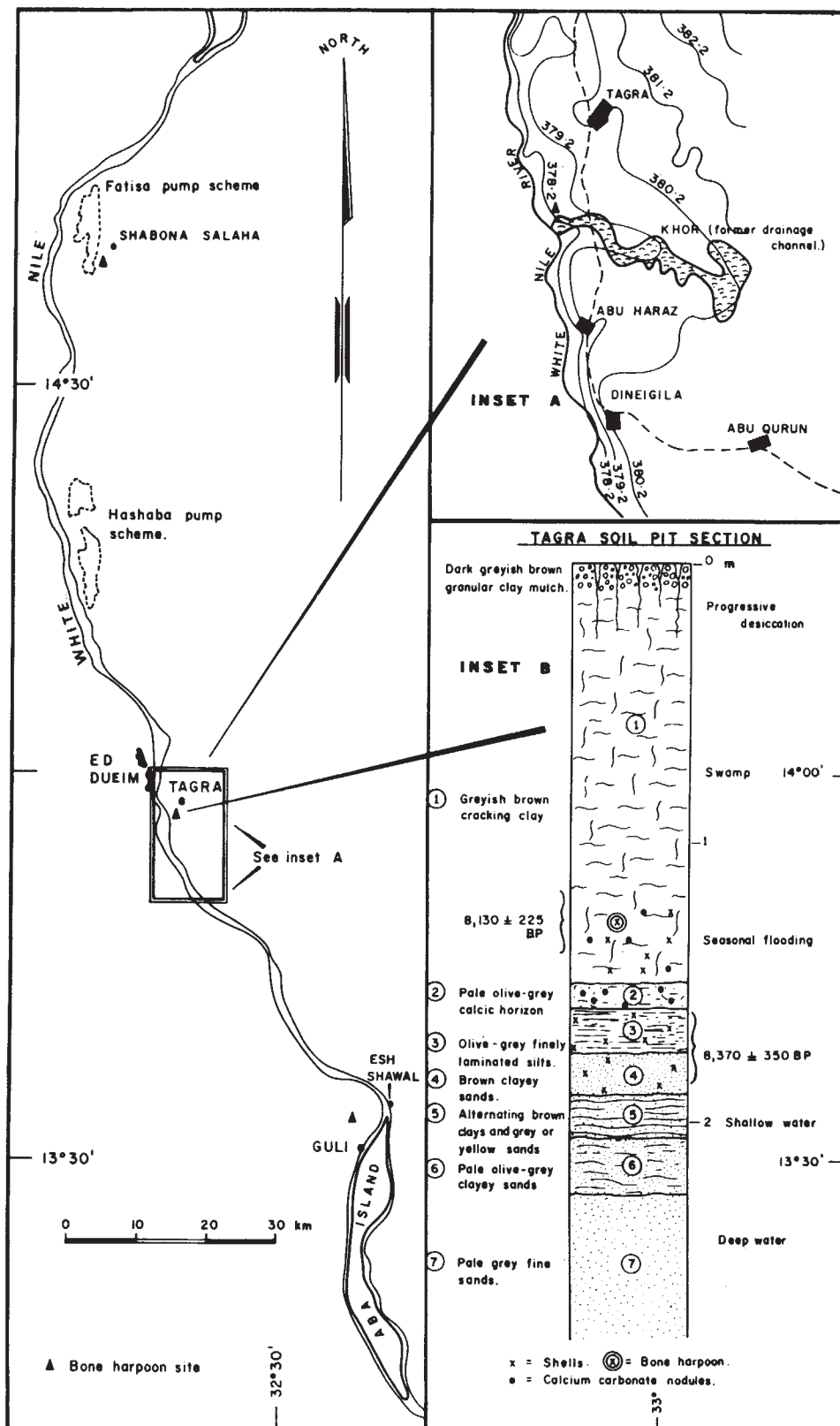


FIG. 1 Location of the three sites in Central Sudan, with inset showing section of soil pit at Tagra.

level. The limonite bands between gleyed silt laminae also suggest an alternation of oxidising and reducing conditions associated with seasonal deposition in a shallow littoral environment.

The deposition of clay, the calcic layer, the absence of permanent-water mollusca and the signs of human activity are also consistent with the cessation of permanent water

conditions and a change to seasonal inundation. The clay indicates inundation by slow moving water carrying only fine sediment. The site may have been subject to the type of annual flooding and partial drying which now occurs in 'toich' soils which adjoin rivers or 'khors' in the permanent swamps of the Sudd region in the southern Sudan⁶. A zone of carbonate accumulation, which may mark the depth of

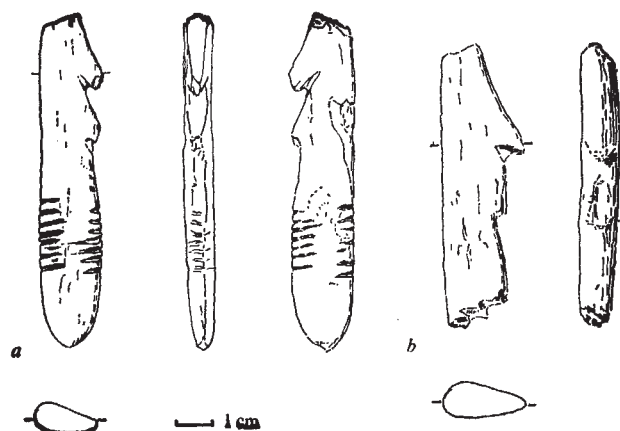


FIG. 2 a, Tagra bone point fragments after joining; b, Guli specimen (drawn by R. Vermilion).

seasonal drying, is common in 'toich' soils and the horizontal calcic band in the Tagra profile may be analogous. If the permanent, dry season water level of the White Nile was about 376–377 m, the flood season levels would have created 'toich' conditions at this site during the whole period of clay deposition.

The recession of the White Nile and the end of clay deposition by seasonal flooding at this site was governed by the down-cutting of the Blue Nile and consequent lowering of the White Nile⁷. The White Nile fell by about 4 m within the past 6,000–8,000 yr, sufficient to account for a lowering of the level of permanent water, that is dry season level, at this site from about 376–377 m to the present pre-dam level of 372.2 m. The effect of the Jebel Aulia dam when full is to raise the White Nile close to its prehistoric dry-season level of 8,000 yr ago.

Guli site

A second fragment of barbed bone point, heavily encrusted with calcium carbonate, was found on a small sandy island in the White Nile swamps near Guli (Fig. 1) at 13°34'N, 32°34'E at an estimated elevation of about 378.5 to 379 m, that is about 1 m above the present high water level caused by the Jebel Aulia Dam (377.8 m). Before completion of the dam, the maximum White Nile level, estimated by interpolation between the Ed Dueim and Rabak gauges, was about 375.6 m and the minimum about 373.0 m. The island is part of an old point bar in the large area of swamps and point bars, about 7 km wide, on the west side of the river opposite Esh Shawal. The bone tool was found accidentally while sampling a dense deposit of *Pila wernei* shells (0–35 cm deep) for purposes of radiocarbon dating. The spoil from a drainage ditch across one corner of the deposit revealed abundant potsherds with impressed fish-spine decoration, fossilised and subfossil fish bones, and subfossil *Pila* shells, indicating that the shell deposit was associated with an old occupation site. The *Pila* shells were predominantly mature specimens averaging 30–50 mm, with large specimens up to 90 mm long. The whole shell assemblage was evidently not a natural one and this suggests that they were collected and brought to the occupation site as food and/or bait. A sample of shell from a depth of 30–35 cm in the midden gave an age of $5,500 \pm 90$ yr BP (or 3530 ± 90 B.C.) (SUA-211) thus suggesting that the Guli site is contemporaneous with the "Khartoum Neolithic" tradition as represented at Esh Shaheinab and other sites north of Khartoum.

Bone point fragments

The Tagra bone point fragments (Fig. 2a) have been cleaned and the carbonate removed by the Technical Department of

the British Museum (Natural History) to the staff of which our thanks are gratefully acknowledged. After the two fragments are joined, the specimen is seen to comprise the butt end and two partially damaged barbs, both on the same lateral edge. The distal end of the specimen is missing but there is no noticeable convergence of the lateral edges so that it is likely to have been furnished with at least one additional barb. The existing length is 91 mm; the maximum breadth at the upper barb is 18 mm and, at a distance of 21 mm from the proximal end, the maximum breadth is 16 mm. In section it is a flattened ellipsoid, more or less the same thickness throughout its length — 7.5 to 7.0 mm.

The specimen is made from a longitudinally split fragment of long bone from a large mammal, rubbed smooth and polished on a grindstone. This smoothing and grinding have been insufficient to remove entirely evidence of the cancellous bone on the interior surface. The barbs have been made by working on each face opposed V-shaped grooves; these were set obliquely to the long axis and deepened so as to intersect at the edge. The intersection was then worked back by further transverse rubbing to deepen and enlarge the barb. The backs of the barbs are slightly concave, as in many of the "Early Khartoum" examples, and although the tip ends have been broken away, they may similarly have been given a reverse curve, for the beginnings of such can be detected on the upper of the two surviving barbs.

As in the uniserially barbed bone points from "Early Khartoum", the Tagra specimen has also been provided with seven obliquely cut grooves between 40 mm and 21 mm from the proximal end. These grooves are believed to serve as an anchor for the binding that secured the point to the shaft so that, since the butt carries no perforation, the specimen is technically the non-detachable point of a spear rather than a harpoon.

The Tagra specimen closely resembles the "Early Khartoum" points in general dimensions, nature of barbs and grooving of the rounded butt end. Only a small number, however, out of more than 270 fragments at "Early Khartoum" have a flattened, ellipsoidal cross-section, most being circular or elliptical in section. Variability of this kind is not unexpected between independent settlements of this kind over 180 km apart. There is, however, no indication that the Tagra point was associated with any living site debris; it seems to be an isolated find that had, probably, been lost in use away from the immediate settlement area.

The specimen from Guli (Fig. 2b) is the central portion of a similar uniserially barbed bone point; the distal and proximal ends are both missing. It is 76 mm long, 22 mm broad across the first barb and the thickness varies between 9.0 mm and 8.0 mm. It is made in a similar way from a segment of longbone rubbed down on a sandstone grinder. The upper of the two barbs is complete but only the basal part of the second, adjacent to the stem, is preserved. The back of the complete barb exhibits the concavo-convex profile characteristic of the bone points and harpoons from both "Early Khartoum" and Esh Shaheinab. The section across the surviving barb is a flattened ellipse, similar to that of the point from Tagra. The missing distal portion probably ended in a point as it can hardly have been either long or broad enough to accommodate another barb unless this was of a vestigial nature. Similarly, there is no indication from the remaining length of the proximal end that there were ever more than two barbs. Although rare, forms with only two barbs are known from both "Early Khartoum" and Esh Shaheinab. The specimen is coated with a carbonate skin which has not yet been removed, but there is nothing to lead one to suspect that the butt end bears groovings characteristic of points of "Early Khartoum" type. Neither is the surviving portion lugged and perforated as were the Neolithic harpoons, however, since the butt itself is missing, it is not possible to be certain that it may not have been pierced for

the attachment of a line, though this seems unlikely.

The Guli example resembles more closely, therefore, the "Early Khartoum" type of bone point than it does the perforated and lugged type of harpoon from Esh Shaheinab, although the radiocarbon determination suggests that it is more closely related in time to the Neolithic than the "pre-Neolithic". Stylistic preferences and time differences between river bank settlements at this time are surely to be expected, as also is variability due to differences in the local availability of specific resources at the different sites and so in the equipment with which these were exploited. The Neolithic bone harpoon may well be related to hippo-hunting from dugouts or reed boats, as Arkell has previously hinted (ref. 2, page 31). The barbed bone point, on the other hand, is more probably a spear for Nile perch and mud fish as well as for land mammals; barbed spears and harpoons have a very long history in the Nile basin⁸ and in the west African Sahel⁹.

The pottery from Guli shows affinities with the Khartoum Neolithic impressed wares though there are no sherds of "Burnished Dotted Wavy-Line" or opposed triangle impressed pottery in the collection. The majority of sherds seem to come from well-fired, deepish spherical bowls with inturned rims, the upper half at least of the exterior of the pot, and sometimes the lip, being decorated with horizontal rows of impressed dots, some executed with a comb (cat fish spine or stamp) and others again with a rolled cord or grass roulette (compare ref. 2, plate 29:2). Rocker stamp decoration occurs on the interior of one sherd and the rim of a second. Another shows two rows of stamping on the rim and parallel grooving below; the sherd is also perforated. Another rim sherd shows a stamped chevron design such as occurs rarely also at Esh Shaheinab. These sherds are comparable also to the Khartoum Variant pottery from the 2nd Cataract and that from the Khashm el Girba region on the Upper Atbara which is similarly unburnished¹⁰. The pottery confirms in general, therefore, the age determination of the Guli midden and bone point fragment.

Age determinations on bone, shell and charcoal from the pre-Neolithic settlement at Shabona have not yet been received, but the similarity of the barbed bone points from this site (where the flattened section is more common than the circular form) and those from "Early Khartoum" to the Tagra specimen, suggest that this particular industrial tradition most probably belongs in the seventh and sixth millennia bc. The "Early Khartoum" tradition had been replaced by that of the Khartoum Neolithic, which grew directly out of it, by the middle of the fourth millennium bc on the evidence of the dates from Esh Shaheinab and Guli, but its lower limit has yet to be determined. There

is every indication that it was a tradition of long duration evolving out of the local late Palaeolithic and early epi-Palaeolithic industries, through the gradual development of a specialised waterside technology and the acquisition at different times in its history of pottery and other improved cultural traits.

The only other early site on the Nile yielding barbed bone points is the fishing camp of Cat Fish Cave in Upper Egypt (22°43'N, 32°07'E) dating to before 6400 b.c.¹¹. No pottery was found here but the points bear only general resemblance to those of the "Early Khartoum" tradition. There is no pottery either at the epi-Palaeolithic site of El Kab (c. 25°15'N, 32°53'E) dating to 6400 ± b.c., neither, however, are there bone points¹². Pottery is again absent from the epi-Palaeolithic sites found by Wendorf¹³ in the Fayum depression and dating between 5190 ± 120 and 6150 ± 130 b.c. A specialised form of barbed point made from a cat fish spine is associated with these small fishing camps. The absence of pottery from these seventh millennium sites in Egypt suggests, therefore, that the Tagra barbed bone point might belong to a similar early Holocene local Sudanese tradition that had not yet developed the art of pottery making. On the other hand, if the dates from Shabona prove to be coeval with that from Tagra, the possibility that the characteristic "Wavy-Line" pottery and other wares of "Early Khartoum" type were an early and independent development on the Upper Nile or in the Sahara, as Arkell has suggested¹⁴, becomes much more likely.

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Localisation of a fibroblast growth factor and its effect alone and with hydrocortisone on 3T3 cell growth

Denis Gospodarowicz

The Salk Institute for Biological Studies, PO Box 1809, San Diego, La Jolla, California 92112

FGF, a polypeptide found in brain and pituitary, provokes the initiation of DNA synthesis by resting 3T3 cells. When glucocorticoids are present FGF stimulates DNA synthesis as effectively as serum.

MULTIPLICATION of animal cells *in vitro* requires certain macromolecular factors which are usually found in serum. Since none of these factors, except for somatomedin A^{1,2} had

been isolated until recently, their mode of action has remained unclear. It is also unclear whether one or more of these factors is required to initiate division *in vivo* or *in vitro*. It has been suggested³⁻⁷ that the pituitary gland is a source of growth factors *in vivo*. Bovine pituitary glands have yielded an ovarian growth factor which controls the division of an ovarian cell line⁴. Other reports describe the presence in impure preparations of pituitary hormones such as thyroid stimulating hormone and luteinising hormone of factor(s)

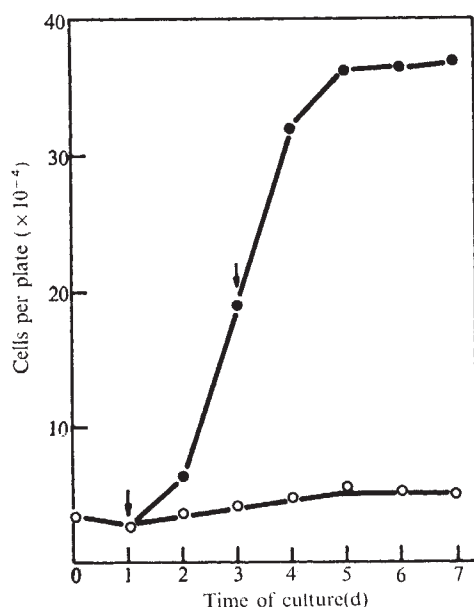


FIG. 1 Stimulation of division of resting 3T3 cells by FGF. 3T3 cells were plated in Dulbecco's modified Eagle's medium (DME) in the presence of 10% calf serum (4×10^4 cells per 60×15 mm plate). Eight hours later the medium was changed to DME with 0.4% calf serum (day 0). On day 1 and day 3 (arrows), the FGF was added (50 ng ml^{-1}). The number of cells per dish was counted every day and plotted against time both in the presence (●) and the absence (○) of FGF.

able to stimulate growth of chondrocytes⁵ or 3T3 cells^{6,7}.

To ascertain whether or not the pituitary is the sole source of growth factors, crude extracts of a variety of bovine organs were tested for their ability to stimulate division of resting 3T3 cells. The highest concentration of fibroblast growth factor (FGF) was found in the brain. Pituitary extract was five to ten times less potent. Although FGF has been purified from the pituitary⁸, the effort to purify it from the brain has been impeded by the high concentration of basic polypeptides of low molecular weight present in brain extracts. These polypeptides remain associated with the FGF making its purification from brain more difficult than from pituitary.

Studies of the control by purified FGF of the division of resting 3T3 cells maintained at low serum concentration have shown the stimulation of DNA synthesis by FGF to be less than that induced by a high serum concentration. For maximal DNA synthesis, hydrocortisone (a glucocorticoid) is required. This requirement is similar to that of an ovarian cell line³, which requires dexamethasone or hydrocortisone for full cell division. In serum-free medium, FGF plus hydrocortisone stimulates DNA synthesis in a manner comparable with serum.

Localisation of activity

Growth factor activities of crude extracts of the following organs from freshly slaughtered cows were compared: heart, liver, muscle, pituitary, brain, placenta (cotyledon), adrenal cortex, adrenal medulla, ovary and lung. Each tissue was homogenised at 4°C in $0.15 \text{ M } (\text{NH}_4)_2\text{SO}_4$ in a Virtis homogeniser. The pH was adjusted to 4.5 and the homogenate was allowed to stand in the cold. After 2 h the homogenate was centrifuged and the supernatant was dialysed against deionised water overnight. The extract was then centrifuged again; the supernatant was lyophilised and stored for use.

The 3T3 cells used were grown in DME (Dulbecco's modified Eagle's medium) supplemented with 10% calf serum

(Colorado Serum Co. Laboratories) and incubated at 37°C in a CO_2 and humidity controlled incubator. The cells were collected routinely using trypsin (0.25% in phosphate-buffered saline). Aliquots of the washed cells were plated onto plastic tissue culture dishes (Falcon). The serum concentration was decreased to 0.4% to make the cells enter a resting stage (Fig. 1). DNA synthesis was assayed quantitatively by measuring ^3H -thymidine incorporation into trichloroacetic acid-precipitable material.

When brain or pituitary extracts were added to cultures of resting 3T3 cells, DNA synthesis was enhanced in proportion to concentration (Fig. 2). In contrast, muscle and kidney extracts were inactive except when high concentrations ($2.5 \mu\text{g ml}^{-1}$) of protein were present. The slight growth factor activity of kidney extracts was consistent with the activity to be expected due to the presence of serum in the kidney at the time of extraction.

The effects of crude extracts of diverse tissues is presented in Table 1. The incorporation of ^3H -thymidine by 3T3 cells is clearly much higher in the presence of crude brain or pituitary extract than in the presence of extracts from any other tissue tested. The slight degree of stimulation by extracts from other tissues can, in certain cases, be attributed to their serum content. For example, if liver is perfused with saline before extraction most of the stimulatory effect disappears.

Brain extract was a more potent stimulant of DNA synthesis than pituitary extract (Fig. 2). The minimum concentration of brain extract which stimulated ^3H -thymidine

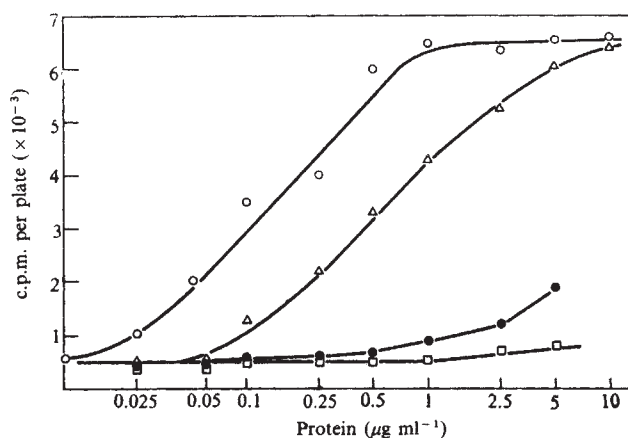


FIG. 2 DNA synthesis in 3T3 cells in response to various concentrations of diverse crude extracts. 3T3 cells were plated in DME with 10% calf serum (4×10^4 cells per 60×15 mm dish with 5 ml of medium per dish). Eight hours later the medium was removed and replaced by DME with 0.4% calf serum. Twenty-four hours later the same washing was repeated after which the cells were maintained for 3 d without changing the medium. On day 3, various concentrations of samples dissolved in the presence of 0.5% crystalline bovine albumin (to minimise nonspecific adsorption) were added to the cultures. Samples were added in fluid volumes ranging from 25 to $100 \mu\text{l}$. Sixteen hours later, $50 \mu\text{l}$ of DME containing $10 \mu\text{Ci}$ of ^3H -thymidine (^3H -methyl, NEN) and $6 \mu\text{g}$ of cold thymidine were added to each dish. After 11 h incubation at 37°C the dishes were washed once with a buffered salt solution, after which 2 ml of 0.5 N NaOH was added and the dishes were further incubated for 8 h at 37°C . DNA was then precipitated by adding trichloroacetic acid (TCA) to a final concentration of 10%. The precipitates were collected on Whatman GF/C glass fibre filters, washed twice with 10% TCA and counted (scintillation counter with toluene-PPO-POPOP solution) as radioactivity incorporated in DNA. The incorporation of ^3H -thymidine was $650 \text{ c.p.m. per dish}$ in controls. All points were done in triplicate. Standard errors did not average more than 10% of the mean. ○, Brain; Δ, pituitary; ●, kidney; □, muscle.

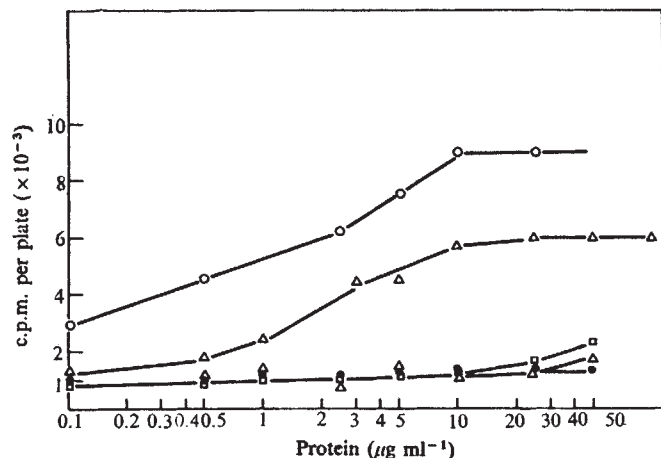


FIG. 3 DNA synthesis in C6 glial cells in the presence of various concentrations of diverse crude extracts. C6 cells were grown as described for 3T3 cells. Cells were plated in DME plus 5% calf serum (6×10^4 cells per 60×15 mm dish with 5 ml of medium per dish). Eight hours later the medium was removed and replaced with DME plus 0.2% calf serum. Twenty-four hours later the same washing was repeated after which the cells were maintained for 3 d without changing the medium. On day 3, various concentrations of samples dissolved in the presence of 0.5% crystalline bovine serum albumin (to minimise nonspecific adsorption) were added to the cultures. Samples were added in fluid volumes ranging from 25 to 100 μ l. Determination of ^3H -thymidine incorporation was as described in Fig. 2. The incorporation of ^3H -thymidine was 850 c.p.m. per dish in controls. All points were done in triplicate. Standard errors did not average more than 10% of the mean. ○, Brain; Δ, pituitary; □, liver; ●, adrenal cortex; Δ, ovary.

incorporation was 25 ng ml⁻¹ compared with 100 ng ml⁻¹ for pituitary extract. This is a comparison of pituitary extract and extract of whole brain. If growth factor activity in the brain can be localised to some small region, the specific activity of that region will be much greater than that of the more 'dilute' whole brain preparation and, accordingly, much greater than that of the pituitary extract.

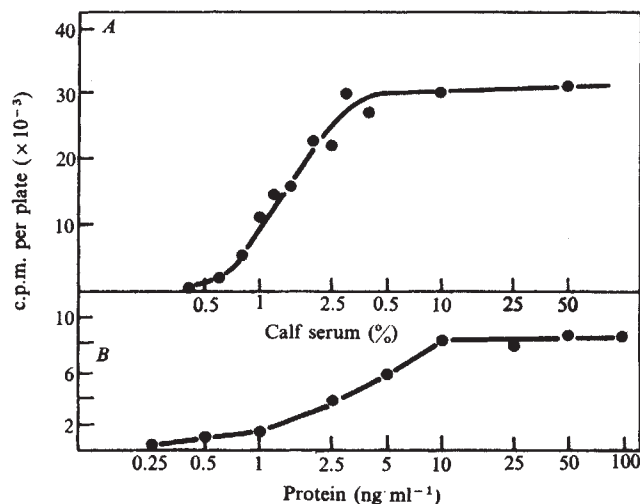


FIG. 4 DNA synthesis in 3T3 cells in the presence of serum compared with FGF. 3T3 cells were plated and maintained as described in Fig. 2 (4×10^4 cells per 60×15 mm dish). The incorporation of ^3H -thymidine was 600 c.p.m. per dish in controls. A, Effect of increasing concentrations of calf serum on the incorporation of ^3H -thymidine into DNA. B, Effect of increasing concentrations of FGF on the incorporation of ^3H -thymidine into DNA.

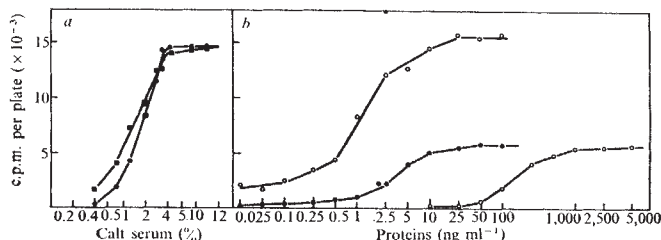


FIG. 5 DNA synthesis in 3T3 cells in the presence of serum, hydrocortisone and FGF. 3T3 cells were plated and maintained as described in Fig. 2 (2.4×10^4 cells per 60×15 mm dish). The experiment was conducted as described in Fig. 2. a, 3T3 cells maintained in the presence of increasing concentrations of serum (●) and serum + 0.5 μ g ml⁻¹ of hydrocortisone (■). The incorporation of ^3H -thymidine was 300 c.p.m. per dish in the controls. b, 3T3 cells maintained in the presence of increasing concentrations of FGF in the presence (○) and absence (●) of 0.5 μ g ml⁻¹ of hydrocortisone. The stimulatory effect of NIH bovine LH-B-8 (bovine luteinising hormone) where the FGF is present as contaminant is shown by the lowest line. The purified FGF is about 200-500 times as potent as that preparation.

To test the ubiquity of the growth stimulating effect of brain and pituitary extracts cell lines other than 3T3 have been used. Figure 3 shows that the growth of C6 glial cells

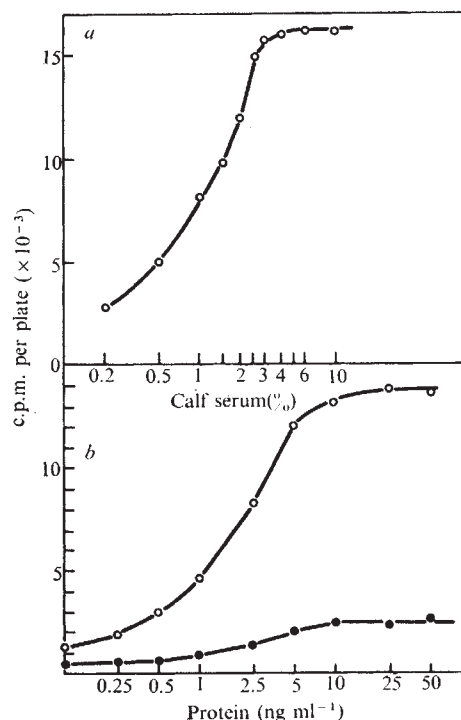


FIG. 6 DNA synthesis in 3T3 cells in the presence of either serum or FGF plus or minus hydrocortisone. 3T3 cells were plated and maintained as described in Fig. 2 (2.4×10^4 cells per 60×15 mm dish). Twenty-four hours before the addition of FGF and hydrocortisone the 0.4% calf serum medium was removed and replaced with DME containing 0.25% crystalline bovine serum albumin and 0.5 μ g ml⁻¹ of hydrocortisone; in some dishes, hydrocortisone was not added. Twenty-four hours later FGF and serum were added to the dishes in graded concentrations and the experiment was conducted as described in Fig. 2. The incorporation of ^3H -thymidine was 300 c.p.m. per dish in the controls. a, Effect of increasing concentrations of calf serum on the incorporation of ^3H -thymidine into DNA of 3T3 cells maintained in the absence of serum and in the presence of hydrocortisone. b, Effect of increasing concentrations of FGF on the incorporation of ^3H -thymidine into DNA of 3T3 cells maintained in the absence of serum and in the presence (○) or absence (●) of hydrocortisone.

TABLE 1 DNA synthesis in 3T3 cells maintained in the presence of increasing concentrations of various crude extracts

Crude extracts	Protein concentrations (ng ml ⁻¹)			
	100	250	1,000	5,000
Brain	4,600	8,500	10,200	9,500
Pituitary	2,500	5,100	7,600	10,200
Liver	680	1,000	1,860	3,600
Perfused liver	720	690	1,250	1,820
Cotyledon	620	650	1,200	1,150
Heart	610	670	750	1,600
Muscle	625	810	925	1,750
Ovary	650	710	1,200	1,560
Adrenal cortex	670	925	970	1,920
Adrenal medulla	580	570	420	750
Kidney	630	825	1,250	1,850

Results are expressed in c.p.m. of ³H-thymidine incorporated into DNA. 3T3 cells were plated as described in Fig. 2 (32,000 cells per 60 × 15 mm dish) and ³H-thymidine incorporated into DNA was assayed as described in Fig. 2. The incorporation of ³H-thymidine was 630 c.p.m. per plate in the controls. All points were done in triplicate and standard errors did not average more than 10% of the mean.

is also stimulated. This cell line was chosen because the cells remain differentiated in culture, as demonstrated by the existence of a functional nor-adrenaline receptor⁹ and by their secretion of S₁₀₀ protein, a specific marker for glial cells¹⁰. As with 3T3 cells, C6 cells respond to crude brain and pituitary extracts by an increase in DNA synthesis. However, C6 cells are less sensitive to the extracts than are 3T3 cells. Furthermore, the maximum level of stimulation produced by brain extract is twice that produced by pituitary extract. Extracts of other tissues had no effect (Fig. 3). I now have evidence that crude extract of brain or pituitary can induce division not

only of 3T3 and C6 cells but in primary cultures of glial cells as well. The growth factor activity in pituitary and brain is then not restricted to a single cell type.

Purification and activity

The purification of FGF from pituitary glands (described in full in ref. 8) is briefly as follows. Bovine pituitary glands are extracted at pH 4.5 with 0.15 M (NH₄)₂SO₄. Addition of HPO₄ to pH 3.0 removes an inactive fraction. FGF activity was precipitated between 2.34 and 3.8 M (NH₄)₂SO₄ at pH 7.0 and further purified by carboxymethylcellulose chromatography. The activity was adsorbed from 0.01 M potassium phosphate, pH 5.9, and eluted with 1 M NaCl and 0.1 M potassium phosphate, pH 5.9. Further purification was achieved by chromatography on Sephadex G-75 and Sephadex SE C-50. The yield of purified FGF averaged 1 mg kg⁻¹ of pituitary. Acrylamide gel electrophoresis at pH 4.5 (ref. 11) revealed a single band. Contamination by pituitary hormones was negligible.

As mentioned earlier, purification of the FGF from the brain is more difficult and has progressed more slowly. An effort is under way to purify FGF from the brain in order to determine whether or not it is identical to the FGF purified from the pituitary.

When purified FGF was added to resting 3T3 cells maintained at low serum concentration, they entered a division cycle as evidenced by an increase in cell number compared with controls, and as demonstrated by autoradiography (Fig. 1).

The initiation of DNA synthesis by 3T3 cells maintained in the presence of 0.4% calf serum in response to FGF was assayed quantitatively by measuring ³H-thymidine incorporation into trichloroacetic acid-precipitable material. As Fig.

TABLE 2 Degree of stimulation or inhibition by steroids on the incorporation of ³H-thymidine into DNA of 3T3 cells maintained in the presence of FGF

Steroids	Conc. (μg ml ⁻¹)	³ H-thymidine (c.p.m.)	Inhibition* (%)	Steroids	Conc. (μg ml ⁻¹)	³ H-thymidine (c.p.m.)	Inhibition (%)	Stimulation† (%)
Δ ⁴ -Androstene-3, 17-dione	0.1	3,521	62	Δ ⁴ -Pregnen-21-ol-3, 20-dione	0.1	13,803		149
	1.0	2,098	78		1.0	14,358		156
	10.0	30	100		10.0	2,324	75	—
Δ ⁵ -Androstene-3β, 17β-diol	0.1	3,198	65	Δ ⁴ -Pregnen-11β, 21-diol-3, 20-dione	0.1	15,202		163
	1.0	2,898	69		1.0	14,573		157
	10.0	1,534	84		10.0	9,098	—	—
Δ ⁴ -Androsten 17β-ol-3-one	0.1	4,478	52	Δ ⁴ -Pregnen-21-ol-3, 11, 20-trione	0.1	3,129	62	
	1.0	1,805	71		1.0	3,300	65	
	10.0	(-)-656‡	100		10.0	(-)-716	100	
Δ ⁵ -Pregnen-3β-ol-20-one	0.1	924	90	Δ ⁴ -Pregnen-11β, 17α, 21-triol-3, 20-dione	0.1	19,937		215
	1.0	(-)-694	100		1.0	21,018		226
	10.0	(-)-525	100		10.0	19,134		206
Δ ⁴ -Pregnen-3, 20-dione	0.1	8,264	11	Dexamethasone	0.1	24,278		261
	1.0	7,008	25		1.0	24,481		263
	10.0	(-)-286	100		10.0	18,985		204
17β-Oestradiol	0.1	4,088	56	FGF	50 ng ml ⁻¹	9,283		
	1.0	2,097	78	Serum	8%	26,741		
	10.0	(-)-680	100					

3T3 cells were plated and maintained as described in Fig. 2 (3.2 × 10⁴ cells per 60 × 15 mm dish). On the day of the experiment, FGF (50 ng ml⁻¹) plus the different steroids were added to the dishes. The steroids were added in a final volume of 25 μl of ethanol. Serum was added to a final concentration of 8%. Thymidine incorporation was measured as described in Fig. 2 and the radioactivity incorporated into DNA in the presence of FGF was compared with that obtained in the presence of FGF plus steroids and in the presence of 8% serum. The incorporation of radioactivity in controls was 800 c.p.m.; this value has been deducted from the values obtained with FGF, FGF plus steroids and 8% serum.

* Inhibition values are expressed as percentage of the difference between FGF and control levels with control levels (800 c.p.m.) and below defined as 100% inhibition.

† Stimulation values are expressed as percentage of the FGF stimulation.

‡ In some cases the radioactivity incorporated into DNA when FGF plus steroids were present was less than that of controls; this is indicated by the sign (-).

4 shows, when increasing concentrations of FGF were added to resting cells they started to make DNA more actively. The minimal effective dose of FGF was about 0.1 ng ml^{-1} and plateau values were $5\text{--}10 \text{ ng ml}^{-1}$ (Figs 4 and 5). Based on a molecular weight of 13,000 (ref. 8) this represents a concentration of $1 \times 10^{-9} \text{ M}$. These concentrations are comparable with the physiological concentrations of polypeptide hormones in serum and are indicative of the potency of the FGF.

The maximal stimulation of DNA synthesis by FGF is about 30% of that observed with optimal serum concentration (Figs 4 and 5). This could indicate either that only 30% of the FGF-stimulated 3T3 cells enter the proliferative phase, or that the serum-containing medium stimulates more complete division, thus inducing additional DNA synthesis. The first hypothesis was rejected since autoradiography indicated that the entire 3T3 population was actively engaged in DNA synthesis when stimulated by FGF. The second hypothesis was explored by seeking factors which can potentiate the effect of FGF. It has been shown for many biological systems that glucocorticoids potentiate (permissive effect) the biological effect of polypeptide hormones¹². Thrash and Cunningham have reported the stimulation of division of density inhibited fibroblasts by glucocorticoids¹³ and Armelin has reported that glucocorticoids enhanced pituitary growth promoting activity⁶. These observations have led me to examine the effect of hydrocortisone and dexamethasone on FGF-stimulated DNA synthesis. Figure 5 shows that when 3T3 cells were maintained in the presence of FGF and hydrocortisone, the degree of incorporation of ^3H -thymidine into DNA became comparable with that found in the presence of serum. Furthermore, hydrocortisone did not potentiate the stimulatory activity of serum except when the serum was present in small concentrations. This can be interpreted as indicating that the concentration of hydrocortisone in serum is optimal to provoke maximal DNA synthesis. However, at low serum concentrations the hydrocortisone concentration is too low to be effective.

Progestogen, androgen and oestrogen were also tested for their permissive effect on the action of FGF. As Table 2 shows, androgen and oestrogen have an antagonistic effect on the incorporation of ^3H -thymidine into DNA. In the presence of high concentrations ($10 \mu\text{g ml}^{-1}$) incorporation could even decline to less than that observed with control 3T3 cells maintained without FGF. Progesterone in low concentrations had no marked inhibitory effect; however, when the concentration was increased to $10 \mu\text{g ml}^{-1}$, ^3H -thymidine incorporation was inhibited completely. This effect of progesterone was interesting because the compound can compete for the receptor site of glucocorticoids¹⁴.

In contrast to oestrogen, androgen and progestogen, glucocorticoids were agonistic with the exception of dehydrocortisone which was antagonistic. As expected from the previous results, dexamethasone and hydrocortisone were the most potent agonists and brought the incorporation of ^3H -thymidine to a level similar to that observed in the presence of high serum concentration.

If FGF and hydrocortisone are the only factors required for the control of 3T3 cell division, serum requirements for 3T3 cells can be replaced by those agents. As Fig. 6 shows, when 3T3 cells are maintained in the presence of 0.2% bovine serum albumin and hydrocortisone ($0.5 \mu\text{g ml}^{-1}$) they enter a resting stage. When FGF is added, DNA synthesis is initiated. The level of stimulation observed in the presence of an optimal concentration of FGF is comparable with that obtained with serum.

In summary, induction of 3T3 cell division requires only

two agents, a polypeptide which has been purified from the pituitary gland and a glucocorticoid-hydrocortisone. Although the nature of the permissive effect of glucocorticoids on FGF activity can only be the object of speculation, glucocorticoids may be involved in the control of one or more components of the FGF effector system. An attractive possibility is that they modulate the receptor site for FGF; thus, in the absence of glucocorticoids, few or no receptor sites would be present, resulting in little activity of FGF. In their presence, the number of active sites would be induced maximally, leading to maximal activity of FGF. A similar hypothesis for the permissive effect of glucocorticoids on the lipolytic activity of luteinizing hormone¹⁵ and thyroid stimulating hormone¹⁶ has already been suggested.

It should be kept in mind that if glucocorticoids are needed for the induction of 3T3 cell division, that requirement does not necessarily apply to all types of cells. For L929 fibroblasts glucocorticoids are inhibitory¹⁷.

Finally, it should be pointed out that in all the experiments only the initiation of 3T3 cell division and DNA synthesis for which I think the FGF associated with hydrocortisone is responsible have been investigated. The results do not exclude the possibility of the presence in serum of other factors which may affect cell plating, attachment and survival^{18,19}. However, the acquisition of a highly purified polypeptide which triggers division of 3T3 cells *in vitro* should facilitate the study of the initial events which commit the cell to divide.

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Seafloor spreading theory and the odyssey of the green turtle

Archie Carr

University of Florida, Gainesville, Florida

Patrick J. Coleman

University of Western Australia, Perth, Western Australia

*Seafloor spreading theory seems to explain why the ancestors of a green turtle (*Chelonia mydas*) subpopulation which now travels 2,000 km from Brazil to breed at Ascension Island, were induced to swim oceanwards for increasing distances during the gradual separation of South America and Africa in the earliest Tertiary.*

A SUBPOPULATION of the green turtle (*Chelonia mydas*) lives on the coast of Brazil but breeds and nests 2,000 km away on Ascension Island in the central equatorial Atlantic¹⁻⁶. The feat of navigation involved in this odyssey has not been explained^{5,7}. A puzzle of equal stature, and of more fundamental importance, arises in trying to account for the initial stages in the evolution of the adaptation^{3,4}. Populations of the genus *Chelonia* have distinctive characteristics—enormous shoulder musculature together with its support, heavy fat deposits, and a peculiar jaw structure. The ecological regimen responsible for these characteristics is evidently the herbivorous feeding habit¹⁻⁸ and the need that this imposed for travel between a protected, shallow water pasture ground and the exposed shores on which suitable nesting beaches develop. Offshore and oceanic islands are likely to have good surf-built beaches and they almost always offer relative freedom from the nest-predators which plague mainland nesting beaches. In spite of these advantages, however, the Ascension colony has established the adaptations in the face of huge difficulties, inherent in the initial stages, which seem to make impossible demands on the process of natural selection. Seafloor spreading theory may bear directly upon this aspect, and it also offers clues to the navigation problem.

Turtles and central Atlantic opening

Marine turtles of *Chelonia* type inhabited the seas between 'North America' and northwestern Gondwanaland by the beginning of the late Cretaceous, about 100 million years (Myr) ago. Because of the cheloniid fossil record in this general area (refs 9-12 with the references provided there), we postulate that these ancient turtles included the ancestors of *Chelonia mydas*, even though its phylogeny cannot at present be reconstructed. (*Chelonia* has been found in Miocene sediments^{9,10}.) The northern coast of South America was a suitable habitat for these early turtles, providing a tropical to subtropical environment. There is fossil evidence that even then the herbivorous habit had evolved and that there was a separation of residence-pasture and breeding grounds¹². Mammalian egg-predators no doubt acted as a limiting factor in turtle ecology at this time, augmenting the advantage in island nesting.

The opening of the equatorial Atlantic¹³⁻¹⁶ (Fig. 1a and b), marking the final separation of South America and Africa at about 80 Myr ago, took place in steps: first, rift valley formation at about 110 Myr ago, or even earlier; second, sporadic but progressive ocean flooding with a strong west-to-east component by 90-80 Myr ago; and finally development of a ridge system which linked the spreading ridges of the North and South Atlantic and made of it a single ocean by 80 Myr ago.

Volcanic piles are a frequent feature of midoceanic ridges, and some may grow sufficiently to emerge as islands. As spreading

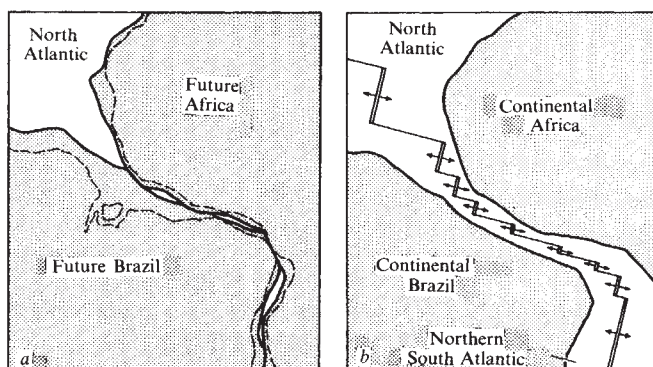


Fig. 1 a, Predrift reconstruction of equatorial 'South America' and 'West Africa', as parts of north-western Gondwanaland, based on the computer-tested bathymetric fit of Bullard *et al.* (ref 23). b, Reconstruction for the time of about 80 Myr ago. A 'Red Sea' connects the northern Atlantic with the younger southern Atlantic and its youthful spreading ridge. Spreading ridge detail is schematic.

continues, they become inactive and are carried outwards and downwards and become seamounts¹⁷, the deepest being furthest from the ridge axis. They are sporadically replaced by new volcanoes on the ridge so that for the continental observer the volcanoes march seawards through time (Fig. 2). The ancestral turtles could thus migrate outwards with the volcanic islands, without the demands on the process of natural selection becoming excessive.

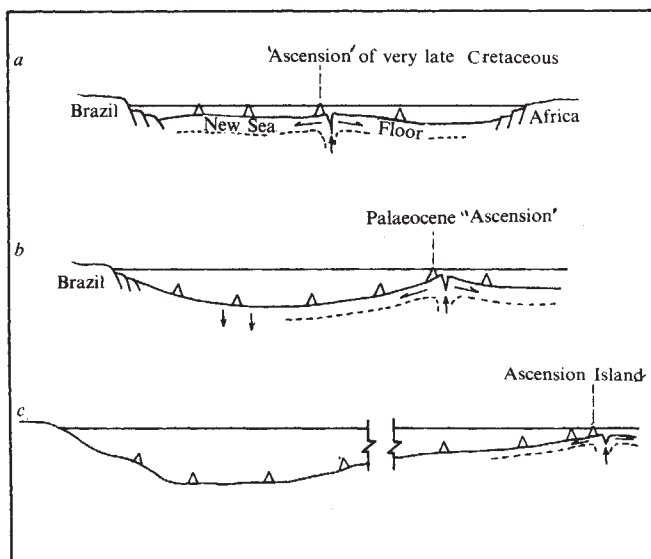


Fig. 2 The progressive appearance, then disappearance, of spreading ridge volcanoes as part of seafloor spreading mechanism. a, Approximately 70 Myr BP; b, approximately 60 Myr BP; c, approximately 1 Myr BP.

Turtle movement into the Brazil–West African channel

Before 100 Myr ago, turtle populations travelled between residence-pasture and breeding grounds along the shores of northern South America. By 90 Myr ago, a corridor had opened at the head of the gulf between Brazil and West Africa in line with the established longshore travel paths. By 80 Myr ago, the corridor was complete, much new coastline had been added, and there was a string of offshore islands. The exploitation of this oceanic channel by the turtles simply required repetitive extension of previous travel paths (Fig. 3). The bearings were WNW–ESE. This is close to a latitudinal course, and could be navigated relatively easily. Locating a particular beach on an island within this narrow sea or along its coasts would involve a longitudinal fix: local currents, rivers, even surfbeat, would offer individual signatures. It seems reasonable to assume that this pattern of travel, constant over a long period of time would become an established, heritable part of the turtle's behaviour.

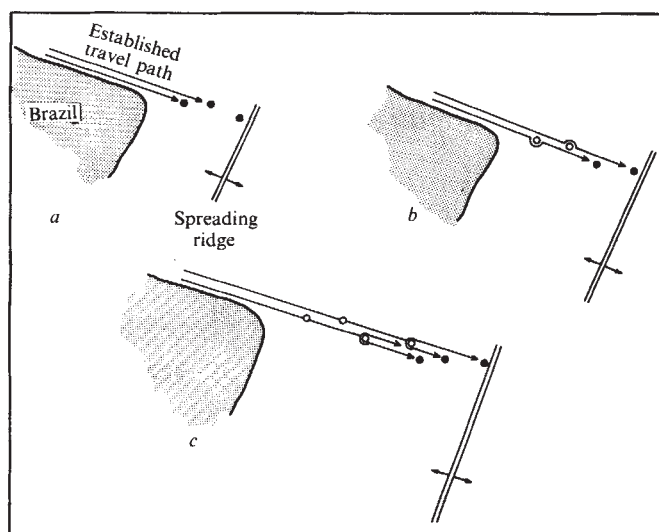


Fig. 3 As seafloor spreading proceeds, through stages *a*, *b* and *c*, new volcanic islands are generated near the ridge crest (double line). Older ones have moved out with the flanks of the ridge and gradually sink until they are submarine (open circle). As a former breeding ground becomes unusable, the turtle swims further on the same, or nearly the same, course.

By 80 Myr ago the Brazil–West Africa sea channel had connected with the northern South Atlantic and its active spreading ridge (Fig. 1*b*). The ridge volcanoes which had formed earliest, were close to those which had arisen or were still arising at the eastern end of the sea channel. For these new ridge islands to become included in the range of breeding grounds it was necessary only that some turtles extend their travel path, that is, simply stay on course, assuming either that the turtle was distracted from its prime target; or that the target had become submerged and unusable. On return trips the turtles would be guided to the final landfall by chemical cues, by surfbeat or even by direct vision. A degree of elasticity in the establishment and recognition of breeding grounds is necessary, but not much more than seems consistent with that found in the modern green turtle¹⁸.

We envisage that by 70 Myr ago, the ancestors of the Ascension Island colony were making seaward breeding migrations of up to 300 km—a figure consistent with the spreading rate of 2 cm yr⁻¹ (refs 14, 19). This process continued (Fig. 3) assuming there was sporadic creation of volcanic islands; Ascension Island is the last and youngest of them (less than 7 Myr old^{19, 20}). What was probably the penultimate island is now a seamount 15 km away, which is submerged to a depth of 1,500 m (ref 21).

We cannot establish the existence of a narrow swathe of seamounts connecting north-east Brazil with Ascension Island,

because the detailed bathymetry is not well known. There is, however, a suggestion of a line of seamounts running south-eastwards from near Recife to about 30° W and then swinging east to Ascension (Fig. 4). This last limb is close to the trace of the Ascension Fracture Zone and its ridge¹⁹. The early Tertiary migration may have been controlled by volcanic islands that arose along the ridge.

Brazil to Ascension

We have argued that, since early in the Cainozoic era, the turtle has had an inherited tendency to swim a particular travel path, roughly WNW–ESE. This path can be followed by using the rising sun as a beacon to stay on latitude, an operation made simpler by the fact that the path is equatorial and the migration period is seasonal; turtles arrive at Ascension from February until May. Thus, for a December departure from the coast of northern Brazil the turtle heads directly into the rising sun (that is, ESE) and does the same every day. At night it rests below the equatorial current or even drifts eastwards with the counter-equatorial current⁵. The journey takes perhaps eight weeks. Because of the northerly drift of the sun at this time of the year, the general path followed from Brazil towards Ascension will describe a gentle arc, convex to the south, of the sort shown in Fig. 4. The required target is the intersection of the travel path with a plume of sensory clues arising from the island. Because of the equatorial current, which at this latitude has a southerly component, the stream line of solubles released from the island is favourably disposed to make the intersection and provide the turtle with a pathway to landfall; the stream line gives a longitudinal fix. The equatorial current also serves another important function, that of carrying the hatchlings, imprinted to Ascension, away from the ecologically unsuitable island⁵. The same requirements probably existed for the early Cainozoic populations, when the separation between Africa and South America was insufficient for development of an equatorial current. Reconstructions of late Cretaceous current systems²² allow us to postulate an equally favourable smaller current system which would perform the same functions during the early stages of the migration to the islands.

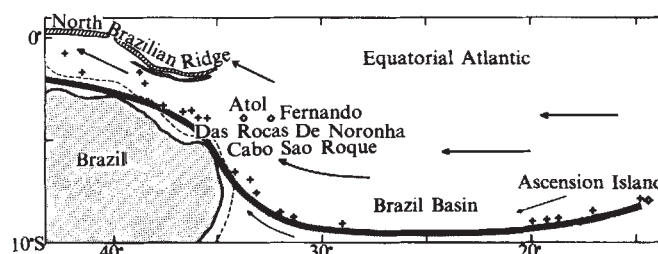


Fig. 4 The western equatorial Atlantic. The arrows indicate the generalised direction of the Equatorial Current (for the northern hemisphere summer). A possible line of seamounts (there are others) is indicated by the vertical crosses. Linking these is a hypothetical migration path (heavy pattern, still to be tested) taken by the Ascension turtles from Brazil to Ascension Island.

This navigatory scheme is simpler than that of Koch *et al.* (ref 7). In particular, it calls for a smaller plume from Ascension, in keeping with the size and nature of the island, and allows for recognition of Ascension emanations, as a new element, against the African background or 'noise' with which the equatorial current is loaded (the Congo contribution alone is massive). If the sun, in combination with chemoreceptive detection of the Ascension plume, is used as a navigational beacon, there would seem to be a corollary which can be examined: turtles from northern parts of the resident grounds would depart first and those from southern parts (south of Recife) would depart later. This is a necessary consequence of the northerly swing of the sun during the visitation period.

Inheritance of behaviour

We suggest that the process of racial learning is of the repetitive, stepping-stone type, which requires no radical change in behaviour at any point. The hypothesis puts the migration to Ascension Island in an evolutionary framework by allowing selective and adaptive processes to operate over a great period of time. Indeed, the period of time may be too long to be acceptable to some. If we are right it means that an extant species has inherited a vital behaviour pattern from ancestors that lived 40 Myr ago and more. In purely taxonomic terms these ancestral species were different, and perhaps very different, from *Chelonia mydas*. In the face of this somewhat unpalatable notion, we take comfort in the realisation that species are more than flesh and bones, especially bones. For the Ascension Island colony, we accept the seaward migration drive as an inherited feature that predates other morphological features conventionally used in systematics to define taxa at species and genus levels. We also accept the far reaching implications which lie behind this statement and its preamble, but we do not have space to discuss them here.

This article is the result, and therefore an acknowledgment, of the scholarly opportunities given to one of us (P.J.C.) during a year as Visiting Professor at the Hawaii Institute of Geophysics, University of Hawaii, where the Director, George Woollard, gave special help. A.C. received support from the National Science Foundation.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Uranium in HR8911

IN recent years there has been growing interest in analysing stellar spectra in order to observe *r*-process elements (which are synthesised by neutron capture on fast time scales). Cool Ap stars seem to be the most likely objects to display lines of such elements^{1,2}. We report here preliminary results concerning some anomalies in the spectrum, which were obtained during analysis of the Cr-Eu-Sr peculiar star HR8911 (κ Piscium) and which show evidence for the presence of *r*-process elements in this object.

Four Mount Palomar spectrograms of HR8911 were measured, one of which was at 4.5 Å mm⁻¹ dispersion, and the spectral lines identified from tracings obtained with a Hilger microphotometer. As only about 2/3 of the spectral lines belonged to common elements of A-type stars, we further examined the unidentified lines in the plate at higher dispersions to search for *r*-process elements. We compared the stellar wavelengths and intensities with those listed by Zeidel *et al.*³. It is important to stress that the following results concern a single spectrogram, whereas HR8911 is a spectrum-variable star and cursory inspection of the other plates shows considerable spectral variations. For example, in another plate lines of Fe I 43, usually strong in spectra of A-type stars, are much fainter than in the measured spectrogram.

Of the heavy elements synthesised in *r*-processes⁴ the presence of osmium and uranium has been confirmed in our analysis. All unblended Os I lines with intensity greater than 50 in the intensity scale of Meggers *et al.*⁵ have been found in

the spectrum, together with many blended, and fainter lines, such as those observed in the spectrum of 73 Draconis⁶. Unblended Os II lines have not been observed. Almost all unblended lines of U II with intensity greater than 40 have been found in our spectrogram and the presence of many other blended lines is suspected. In addition, we remark that the two strongest U II lines ($\lambda = 3,859.58$ Å and $\lambda = 3,854.66$ Å) are unblended and clearly visible in the spectrogram.

Furthermore, there is some evidence for the presence of both neutral and singly ionised platinum in the atmosphere of this star, because the four strongest unblended lines of Pt I and some blended lines of Pt II were observed in the tracings. In addition, the strong Pt II ultraviolet line at $\lambda = 3,551.37$ Å is clearly visible in the other plates (10 Å/mm⁻¹ dispersion).

Among the other *r*-process heavy elements, we found no lines of gold, mercury, lead and bismuth. There are, however, some lines coincidental with Ir I lines, the strongest at $\lambda = 3,800.12$ Å. This is unblended and clearly visible in the spectrogram.

In conclusion, it seems that HR8911 shows evidence for *r*-process elements, so it must be added to those Ap stars in which such elements have already been detected. Thus the complex problem of the origin of Ap stars requires a theory which accounts for the presence of these elements in many objects. The hypothesis of a supernova explosion of a nearby star⁷ is an acceptable theory because the nucleosynthesis of the *r*-process elements requires a very high neutron density, such as that found in the interior of a highly evolved star. We notice further that in this case magnetic accretion⁸, could have an important role.

P. GALEOTTI
E. LOVERA

Laboratorio di Cosmo-geofisica del CNR, Torino
Istituto di Fisica Generale dell'Università di Torino,
Corso M. D'Azeglio, 46
10125 Torino, Italy

Received January 22, 1974.

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Maxwellian relative energies and solar neutrinos

THE solar neutrino discrepancy is now regarded^{1,2} as very serious. The general attitude is that the Sun is trying to tell us something but no one is quite sure what. I wish in this note to add to the list of possible *ad hoc* explanations; namely that the scarcity of ⁸B neutrinos reflects a departure of the distribution of relative kinetic energies from the Maxwellian distribution. I have not been able to calculate a cause of the indicated depletion of the high energy tail of relative energies, so this explanation must be limited to the associated effect.

For an ideal gas (which the solar plasma is not) the Maxwellian distributions of single-particle energies can be shown³ to be equivalent to a Maxwellian distribution for the motion of the centre of mass of the summed masses multiplied by a Maxwellian distribution for the relative motion of the reduced mass. Thermonuclear reactions are calculated with the last of these Maxwellian distributions. For a gas having long range interactions, such as the solar plasma, the distribution function cannot strictly be obtained in such a simple way. The particles undergo many-body collisions and the energy assignable to a particular pair of particles is not defined. A powerful theory could calculate a distribution of relative energies, but only by projecting out of the total distribution its two-particle projection. The usual argument is that the average Coulomb energy is smaller than the average thermal energy, so that an ideal gas is a good approximation.

Nonetheless we should perhaps be cautious, because the thermonuclear reactions involve improbable high energy fluctuations. For example, in a recent model⁴ of the solar centre having $T_c = 14.9 \times 10^6$ K the most effective⁵ energy E_0 for the pp reaction is $E_0(\text{pp}) = 4.58 kT$, and the reaction ³He (α, γ) ⁷Be leading to production of ⁸B involves pairs whose average relative energy is $E_0(3, 4) = 17.4 kT$ — very far out on the tail of the distribution. The power for the Sun comes from energies of a few kT , whereas the neutrinos come from nearly $20 kT$; that causes the ⁸B neutrino flux to be strongly modified if the high energy tail of the two-particle distribution is depleted.

To illustrate these effects I have recalculated the Sun assuming all Maxwellian distributions are multiplied by the same factor $\exp f(E)$, where $f(E)$ is a slowly varying function of energy. I find that the ⁸B flux from the Sun depends on d^2f/dE^2 : if $d^2f/dE^2 < 0$, the ⁸B flux is reduced and if $d^2f/dE^2 > 0$ it is increased. The findings are most easily discussed by expanding $f(E)$ in Taylor series as $f(E) = \beta_0 - \beta_1 E - \beta_2 E^2 + \dots$ and ignoring higher terms as having negligible importance near E_0 .

The constant β_0 is set by requiring that the distribution function remain normalised, and is a function of β_1 and β_2 .

The β_1 term has no physical effect although it might seem to have, since it does seem at first glance to reduce the high energy tail. This would be true at fixed T , but to maintain the Sun's luminosity at its known value, its central temperature would have to be increased sufficiently to counteract the effect of β_1 on the solar power. This increase in T raises the ⁸B neutrino flux back to exactly the value it had for $\beta_1 = 0$. Thus β_1 may be ignored as having no effect. For the ⁸B neutrinos to be reduced in a Sun of fixed luminosity, the deviation from Maxwellian must decrease faster than $\exp(-\beta_1 E)$ between $E_0(\text{pp})$ and $E_0(3, 4)$; that is, d^2f/dE^2 must be negative.

Suppose then that $f(E) \approx \beta_0 - \beta_2 E^2$ and imagine that β_2 is a small number so that no great violence is done to the distribution. It is convenient to think of $\beta_2 E^2$ as being $\delta(E/kT)^2$, where $\delta = \beta_2(kT)^2$ is a smallness parameter for the deviation. My calculations show that values in the range $5 \times 10^{-3} < \delta < 10^{-2}$ are adequate to suppress the neutrino flux to Davis's limit¹. Values of δ in this range would mean that the deficiency in the distribution function is only a few per cent at a few kT , and at $E = 10 kT$ the rate of change of the distribution function with energy due to the β_2 term is still only 10% of the Maxwellian rate of change. These values of δ entail negligible corrections to the equation of state but large corrections to the ⁸B neutrino flux.

Using the same⁴ unperturbed model of the solar centre, I find that the ratio of PPI completions to PPII + III is

$$[\text{PPI}/(\text{PPII} + \text{III})] \approx 10.36 \exp(117\delta) - 0.25 \quad (1)$$

and is 10.11 for the unperturbed model. I also calculate that the ⁸B neutrino flux stands in relation to the unperturbed flux as

$$\frac{\Phi_{\nu}({}^8\text{B}, \delta)}{\Phi_{\nu}({}^8\text{B}, \delta=0)} \approx 10^{-100\delta} \quad (2)$$

For the two values $\Phi = 5 \times 10^{-3}$ and 10^{-2} , the ⁸B neutrino flux is reduced by factors of 3 and 10 respectively. The flux of neutrinos from ($e^- + {}^7\text{Be} \rightarrow {}^7\text{Li} + \nu$) is reduced by factors of 1.7 and 3.0 respectively in a straightforward application of equation (1). The ¹³N neutrino flux, which scales as $\exp(-248\delta)$, is reduced by factors 3.5 and 12 respectively. Thus this *ad hoc* suggestion can substantially lower the neutrino detection rate even for sufficiently small value of δ that its value is otherwise almost inconsequential. The only other major consequence that comes readily to mind is that the equilibrium concentration of ³He at the solar centre will have been increased by the factor $\exp(106\delta)$, which is about 2.7 at $\delta = 10^{-2}$.

These suggestions sound implausible because we are led to believe that the Maxwellian must be correct, even in a non-perfect plasma and even at the level of $\exp(-20)$; but the lack of a positive detection of solar neutrinos by Davis¹ is also implausible. I have been unable to treat the difficult aspects of many-body physics involved, so I write in the hope of more authoritative treatment from others.

I thank many colleagues who have urged me to publish this abbreviated account of a more detailed paper I circulated privately in 1971. This research was supported in part by the National Science Foundation.

DONALD D. CLAYTON

Department of Space Physics and Astronomy
Rice University,
Houston, Texas 77001.

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Martian lee waves revisited

SEVERAL of the Mariner 9 television frames show standing-wave patterns in the clouds of the Martian atmosphere near surface features of high relief. These lee waves have been interpreted as high level condensation clouds in which the brightness variations are due to a combination of true lateral variations in the local concentration of condensed material and the shadows cast on lower levels by such scattering material. Here I demonstrate that the variation in slope of the mean scattering layer with respect to the local horizontal produces a photoclinometric brightness variation strong enough to explain the data with the invocation of quite modest relief in the cloud deck. Although some lee wave clouds in the later frames of the mission leave no doubt that they are mainly pressure trough condensations, lee wave clouds in the early dust-storm phase of the mission are probably largely the photoclinometric effect of vertical oscillations of approximately 40 m in the lines of flow past land protuberances. One interpretation of this result yields a wind velocity of 92 m s^{-1} .

I was particularly interested in a striking frame we chose for the preliminary report of the Mariner 9 mission¹; a frame taken with the wide angle camera on orbital revolution No. 18 which showed an approximately sinusoidal brightness variation near the Martian terminator, superimposed on the broader brightness variation due to the angular progression of the Sun with respect to the local vertical. This apparent lee-wave pattern was highly periodic, with wavelength of about 40 km, and a general trend of isophotes running roughly parallel to the terminator. On Earth such patterns would be readily ascribed to the periodic genesis of clouds above the lifting-condensation-level as laminar flow lines rose and fell several times in gradually damped, near adiabatic, harmonic compressions and expansions excited by flow over a promontory of some type. Such an interpretation is readily verified for lee waves observed much later in the mission after the planet-wide dust storm abated².

I interpret the earlier lee wave in a different manner, in terms of the sinusoidal deformation in the laminar flow line corresponding to the mean scattering level in the top of the dust storm itself. The reason for this has nothing to do with the implausibility of the condensation cloud hypothesis, but rests on the demonstrability that the required deformation, giving rise to a corresponding brightness variation through a change of orientation of the normal vector to the cloud layer with respect to the solar direction, is extremely modest and therefore ought to be the dominant effect.

The development of inferred topography on the basis of the brightness distribution in the image of a surface exhibiting diffuse reflection, and a knowledge of the quantitative law of light scattering for the kind of surface under scrutiny, has been called photoclinometry by common agreement over the past few years. (The word 'photoclinometry' is due to J. F. McCauley from Greek roots *photos* and *clinos*.) An operational photoclinometric theory adapted to light scattering properties peculiar to the Moon was worked out by Watson³ several years ago.

As part of the Mariner Mars programme I have been developing a generalised photoclinometric theory for application to Mars. The lee-wave deformation of the top of the dust storm is a test case for that theory, in part because the particular light scattering law is reliably known from the isophotal contours of Mars as seen by Mariner 9 before insertion into orbit¹, and also because the 'surface' in question, as an optically thick cloud top, was devoid of albedo variations. Photoclinometry can be unambiguously applied to the determination of a three-dimensional surface corresponding to a mean scattering level so long as the length over which significant optical thickness develops is small compared to the lateral distance over which significant relief develops (this is considerably less stringent a condition than that such an optically thick distance should be short compared to the actual relief itself). An analytical approximation yielding the most essential information about the relief was developed some time ago and

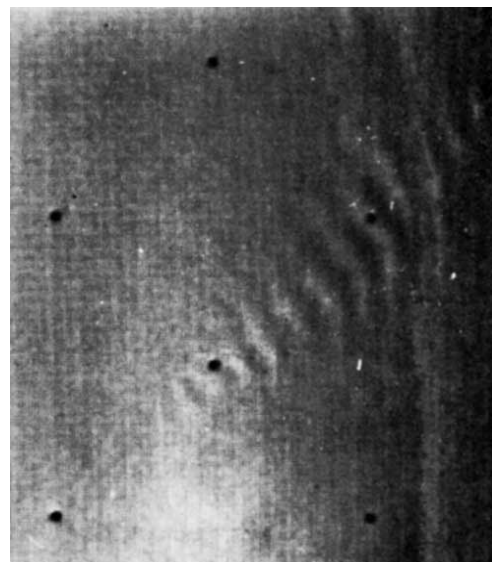


Fig. 1 Lee waves in the top of the great Martian dust storm of 1971, as photographed by Mariner 9. The gradual longitudinal dependence of scattered brightness has been approximately removed, using a computer programme developed by Pat Chavez of the USGS Flagstaff Computer Centre, leaving only the lee-wave effect which may then be greatly enhanced in contrast without partial saturation of the picture.

shows what modest relief is actually implied.

The planet-wide imagery showed that the planet was very nearly a Lambertian reflector under the dust conditions prevailing at the time. I shall use the Lambertian photometric function, $\cos i$, where i is the incidence angle, in the following analysis. I shall also assume that the quantitative character of the hill and dale wrinkling in the upper dust layers of the Martian atmosphere is truly sinusoidal. Consider such ridge lines running parallel to the terminator and in a longitude interval narrow enough for the angle of the solar direction with respect to the local vertical (not surface normal) to be a constant i_0 . If x is a linear distance toward the terminator, then the relative height of the mean scattering level under these assumptions is given by

$$y = A \sin(2\pi x/L)$$

where A is the vertical amplitude of the lee waves and L is the crest-to-crest separation. If i is the angle between the solar direction and the normal to the surfaces of constant dust density in the upper atmospheric layers, then

$$i = i_0 + \tan^{-1}(dy/dx)$$

If the brightness of the surface is described by $B_0 \cos i$, the following brightness variation is derived by simple trigonometric analysis

$$B_0 \cos i = \frac{B_0}{\sqrt{1 + \left(\frac{dy}{dx}\right)^2}} \left[\cos i_0 - \left(\frac{dy}{dx}\right) \sin i_0 \right]$$

Obtaining the derivative from the sine wave 'topography', then

$$B_0 \cos i = \frac{B_0 \cos i_0 - \left(\frac{2\pi A B_0}{L}\right) \sin i_0 \cos\left(\frac{2\pi x}{L}\right)}{\sqrt{1 + \left(\frac{4\pi^2 A^2}{L^2}\right) \cos^2\left(\frac{2\pi x}{L}\right)}}$$

The denominator can be neglected for amplitudes up to 2.3 km. In this range the following simple result is obtained for the relation between the total relative contrast and the total relief

$$\Delta B / \langle B \rangle = 2\pi/L (\tan i_0) \Delta y_{\max}$$

The maximum lee wave contrast is measured in Fig. 1 as 8% at a point where $i = 86^\circ$. The refined ephemeris prediction, which would have placed this point slightly on the dark side of the terminator, was adjusted so that $i = 90^\circ$ occurred just at

zero brightness level. All sources of error considered would probably not change the inferred relief by more than 35%, subject, of course, to the validity of the interpretation. The relief implied by the equation is 40 m.

The basic wind phenomenon at issue does not change under a photoclinoimetric interpretation. If meteorological parameters are to be inferred in part from image measurements, however, the effects discussed here must be dealt with. One of the simplest meteorological interpretations possible, if use is made of the insignificance of the figure of 40 m. in comparison to the length of the lee waves and the cloud height, is obtained by neglecting the crest-to-trough variations in flow velocity in the Bernoulli equation, which is thereby justified. On this view the moving atmosphere can be viewed from a comoving reference frame as an adiabatic oscillator whose expansions and compressions undergo relatively little damping per cycle. The driving force is the buoyancy of the normal atmosphere for vertically displaced cells, from which an oscillator frequency is determined and thus, through the known length of the lee waves, a wind velocity of 92 m s^{-1} . This figure is in fair agreement with other lines of evidence, most notably the sizes of dust particles as inferred from scattering, cloud motions and fluid dynamical considerations, all of which suggest velocities of the order of tens of metres a second. Fuller details will appear elsewhere.

ROBERT L. WILDEY

Northern Arizona University,
Flagstaff, Arizona

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Longitudinal effect in the response of total electron content to magnetic storms

IN a previous study¹ of the response of the ionospheric total electron content (TEC) data to magnetic storms, an average storm time behaviour of TEC for middle latitudes was deduced. On average TEC is enhanced during the first 24 h following the start of a storm, depressed for the next 24 h and near normal through the third 24 h. Another study² has shown a strong diurnal component in TEC behaviour with the maximum enhancement occurring in the dusk sector (1600 to 1800 LT). The present study of disturbed TEC behaviour generally agrees with that reported above but in addition indicates a longitudinal effect in the behaviour of both the storm time and diurnal components of disturbed TEC.

Data on TEC for 21 sudden commencement-type storms during 1969 for six viewing stations at middle latitudes are used to compute the average behaviour both in storm time and local time. No distinction is made regarding the severity of the storms used. The averages are computed as a percentage deviation of TEC from the average TEC on quiet days, calculated for each month using data from the ten magnetically quiet days of each month for each viewing station. In computing the averages the data from each storm are weighted to compensate for nonuniform distribution of storm sudden commencements in UT and are adjusted to compensate for deviation of prestorm values of TEC from the quiet day average TEC (ref. 3). I show the results of the storm time behaviour and the local time behaviour for each of these six stations.

The results of the storm time averages are presented in Fig. 1a for each of the viewing stations in the order of decreasing westward longitude. The usual initial enhancement in TEC is present in all the data during the first 10 to 15 h after the beginning of a storm. The feature of interest is the second enhancement in TEC which occurs between 10 and 30 h after the start of a storm. This second

enhancement is almost nonexistent at the easternmost station (Sagamore Hill) but it becomes more pronounced as one moves in the direction of increasing westward longitude (up in Fig. 1a). It actually becomes the larger of the two enhancements in TEC at the Stanford viewing station.

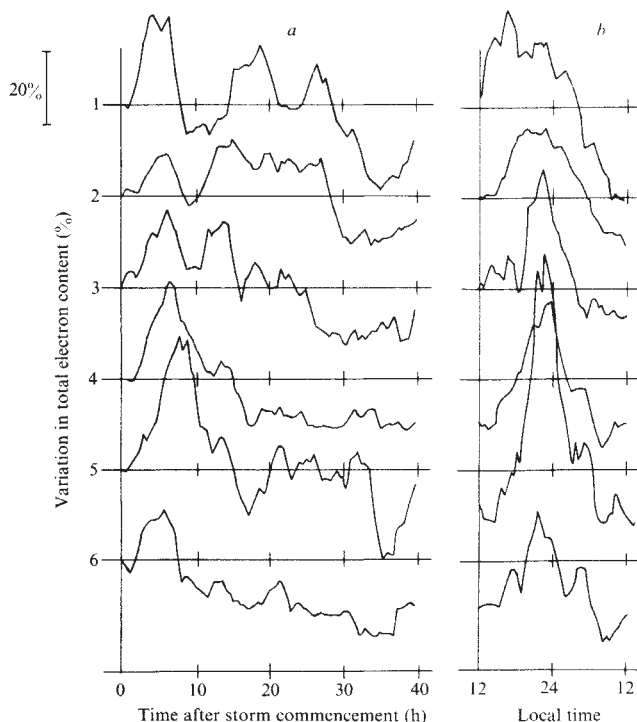


Fig. 1 a, Storm time averages of TEC for 21 storms during 1969 given in order of decreasing westward longitude for: 1, Cold Bay (49.8°N , 160.6°W); 2, Stanford (34.6°N , 125.1°W); 3, Fort Collins (37.0°N , 111.5°W); 4, Urbana (37.0°N , 86.6°W); 5, Rosman (32.6°N , 81.9°W); 6, Sagamore Hill (39.3°N , 71.0°W). b, Local time averages of TEC for 21 storms during 1969 given in order of decreasing westward longitude for the same stations.

The results of the local time averages are shown in Fig. 1b. All the averages show enhancements in TEC occurring during the afternoon and/or evening sectors of the first local time day of the storm. The characteristics of this enhancement also exhibit a longitudinal effect. The westernmost stations tend to have earlier starting and longer lasting afternoon and evening sector enhancements.

These two longitudinal effects are not readily explained by existing storm theories. Some longitudinal variations in the enhancements have been previously reported⁴ but these were attributed to the uneven distribution of storms commencements in UT which would in turn result in a difference in the preferred local start times for each station. The weighted averaging used in this study should reduce this effect considerably.

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ALLEN L. HEARN

Ionosphere Radio Laboratory,
Department of Electrical Engineering,
University of Illinois at Champaign-Urbana

Received February 18, 1974.

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Strong electric fields above an ordinary rain cloud

ON September 6, 1968, in the course of a balloon flight designed to test our theory¹ that magnetospheric electric fields could be measured at balloon altitudes, we observed electric fields much larger than any we have ever observed in fair weather and which we usually attribute to thunderstorms, during the time that a rainstorm occurred under the instrumentation; however, various observations show that no lightning was being generated within any reasonable distance from our observations.

The apparatus is described in ref. 1; it is a set of three electrometers which measure the potential difference between pairs of conducting plates 2 to 3 m apart. Three orthogonal components of the electric field are measured. The apparatus also carries a magnetometer for measuring orientation and is rotated by a motor at one revolution in 6 min. Conductivity at altitude is periodically determined by momentarily shorting pairs of plates together, then allowing them to recharge through the atmosphere.

The balloon was launched at 2055 local standard time on September 5, 1968 (0255 September 6 UT) and reached its design ceiling of 91,000 feet about 1.5 h later. Usually, drifts in the electrometers are large for 0.5 to 1 h after reaching ceiling, possibly because of contamination of the atmosphere by emission of material from the balloon, but the system was quite well stabilised before the beginning of the observations reported here.

The pertinent observations are shown in Fig. 1. Beginning at 0815 UT all three components of the electric field began a marked change, increasing in magnitude to values which were too large for us to measure. During the onset of this event, at 0817 UT, the horizontal component of the field produced by the cloud (the difference from the pre-storm quiet field) was 150 mV m^{-1} at an azimuth of 30° and the vertical field was also 150 mV m^{-1} above the fair weather value. Data from times during which the conductivity was measured have been deleted from Fig. 1 except at 0945 UT where the recharging can be seen on the vertical field plot.

All components of the field remained off scale for about 1 h, except for occasional switching between the two extreme values of the horizontal measurement range caused either by rotation of the instrument or by field variations, and then

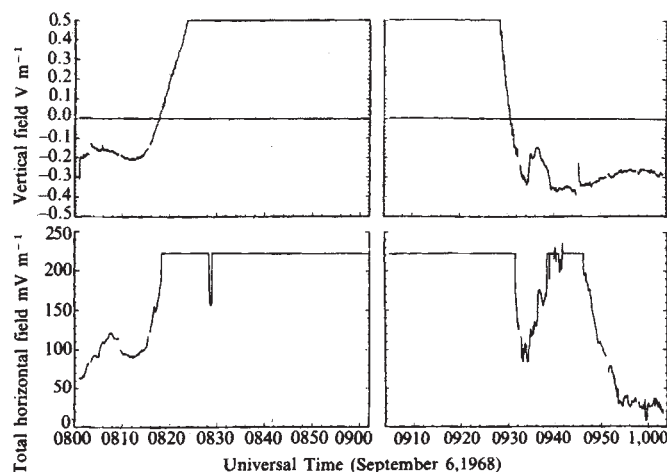


Fig. 1 Electric field variations detected by the balloon-borne instruments.

the field values returned to their 'normal' values over a period of 10 to 20 min. We believe that this phenomenon of switching establishes conclusively that the measurements are real and not the result of a failure in the instrument. Although the electrometers are mainly independent, they do have a common power supply, telemetry and logic system which conceivably might have malfunctioned. But the facts that the horizontal electrometers switch from positive off-scale to negative off-scale as the payload rotates, that they do this one at a time and not together and that the vertical instrument never switches sign, establish the effect as real. At 0932 UT, during the return to normal, the ratio of excess vertical to horizontal fields was 0.85.

The direction of the strong horizontal fields was determined at nine points in the storm, when one or the other of the horizontal axes switched between its extreme values as each of these axes became perpendicular to the fields (Fig. 2). Six of these points gave fields directed generally southwards and the remaining three points indicated westward fields. At the onset of the storm, the horizontal field pointed north, and at the end the field direction was variable, although from 0945 UT to 0951 UT there was an obvious eastward field. These measurements, as well as the brief resurgence of strong fields at the end of the storm, suggest that more than one charged region was present.

During these observations the balloon remained nearly fixed in position about 60 miles north-east of Minneapolis, at $45^\circ 32' \text{N}$, $92^\circ 02' \text{W}$. This position was within easy radar range of the US National Weather Service 10-cm wavelength

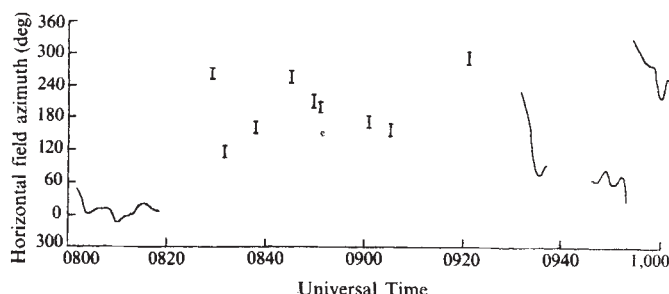


Fig. 2 Horizontal electric field direction.

radar at the Minneapolis—St Paul International Airport and the Weather Service supplied us with the results of their radar observations (Fig. 3). Neither lightning nor thunderstorms were reported within range of the radar in any direction.

Throughout the night, nonetheless, our local v.l.f. monitor recorded strong spheric activity. Observers under the balloon reported no thunder or lightning; thus we attribute the spherics to an intense thunderstorm system that was over the eastern United States at the time, received by means of the enhanced v.l.f. propagation which occurs at night.

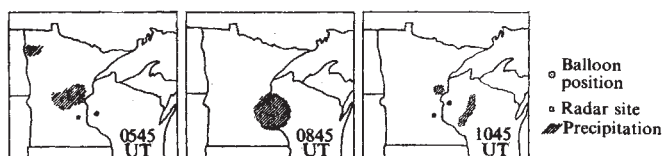


Fig. 3 Precipitation detected by 10-cm radar.

There was, however, a raining cloud some 200 km in diameter, which moved under the balloon when the large fields were observed. The cloud was visible on radar and the balloon recovery team reported rain, as did the tracking team in Minneapolis. The tops of the rainstorm clouds, as reported by radar, were at 18,000 feet. Field-mill measurements from the ground at Minneapolis indicated there was no lightning as the cloud passed overhead (G. Freier, private communication).

Our measurements are consistent with the formation of several bipolar charged regions of the cloud consisting of positive charges located above larger negative charges. The charged condition lasted for about an hour before dissipating.

Conductivity measurements near the end of the storm gave $4\pi\sigma = 0.90 \text{ s}^{-1} \text{ (e.s.u.)}$

at the balloon altitude of 27.6 km. This value is in the general range of fair weather values. Using a model of a charged cloud consisting of two unequal charges at different heights, imbedded in an atmosphere with conductivity varying exponentially with height²⁻⁴, we find that typical horizontal and vertical field data may be fitted by an upper charge of 21 C and a lower charge of -35 C, located 50 km from the balloon. With the measured conductivity, the air-earth current due to the storm is 0.12 A.

We conclude that since we have observed strong electric fields above a cloud which did not generate lightning, there must be some process other than lightning which limits charge growth. This work has been supported by a grant from the National Aeronautics and Space Administration.

MICHAEL WEED
PAUL J. KELLOGG

University of Minnesota,
Minneapolis, Minnesota 55455

Received October 26, 1973.

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Possible new effects in solid state physics

It has been suggested^{1,2} that the electric properties of small enough metallic particles will differ in important ways from those of the bulk material. To test such properties Kreibig and Fragstein³ made nearly spherical silver particles of given radius R , with $30 \text{ \AA} < 2R < 500 \text{ \AA}$. This article is concerned with the prediction of new effects which would be observed if particles of similar size consisting of doped and possibly amorphous semiconductors could be produced. The theoretical and experimental background is discussed by Mott and Davis⁴.

The simplest example of the effect to be discussed is perhaps activated hopping, for instance in an n-type compensated semiconductor. The disorder can localise the electron states and an activation energy W , provided by interaction with phonons, is necessary for transport. For strongly localised states $W \sim N(E) (R_D)^{-1}$, where $N(E)$ is the density of states and R_D is the average separation between donors. For typical specimens of interest $100 \text{ \AA} < R_D < 400 \text{ \AA}$ (ref. 4, page 160) and it may therefore be possible to produce particles such that the particle radius $R < R_D$. In this case $W \sim R^{-3}$ independent of donor concentration. This relationship should be even more easily satisfied in the case of weak localisation, in which case $W \sim (\alpha')^3/N(E)$, where the value of α' is such that $(\alpha')^{-1} > R_D$ (ref. 4 page 41). In this again we should obtain $W \sim (N(E)R^3)^{-1}$. A possible experimental test for such effects is the alternating current conductivity (ref. 4, page 51). The hopping distance R_ω , which makes a maximum contribution at frequency ω , is given by $R = (1/2\alpha) \ln(v_{ph}/\omega)$ where α is of the order of the inverse Bohr radius and v_{ph} is a phonon frequency (ref. 4, page 56). The equation $R = \frac{1}{2} \ln(v_{ph}/\omega)$ can be used to define a critical frequency ω_c for ω , such that for $\omega < \omega_c R < R_\omega$ but for $\omega > \omega_c R_\omega < R$. Thus, if $W \sim R^{-3}$ and $\omega > \omega_c$, then $\sigma(\omega) \sim R^6 \omega^{0.8}$; whereas for $\omega < \omega_c$, $\sigma(\omega) \sim R^{10} \omega$ on the simple theory. This dependence on R and ω could be tested experimentally.

A more dramatic effect seems possible if the current theory of the metal-insulator transition in disordered systems, such as compensated semiconductors, is considered. If the mean square fluctuation of potential is denoted by U_0^2 and J denotes the bandwidth, then the equation $U_0 > 5J$ is believed to be approxi-

mately correct for localisation (ref. 4, page 26). A distance a_E can be defined such that $U_0 = 1/N(E) a_E^3$ (ref. 4, page 26). For a given energy, and $a_E > R$ this equation becomes $U_0 = 1/N(E) R^3$. It follows that for sufficiently small particles the value of U_0 will be large enough for the states to become localised so that a metal-insulator transition will occur. The critical value of R will depend on the energy but will be of the order of R_D at the centre of the band. By the same reasoning it is possible that the region of localised states at the edge of the conduction band in amorphous semiconductors will extend over a greater region of energy in small particles.

I thank N. C. Wickramasinghe for pointing out the importance of studying the properties of small particles.

T. LUKES

Department of Applied Mathematics and Mathematical
Physics,
University College,
PO Box 78,
Cardiff, UK.

Received December 21, 1973.

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Possible origin of Prandtl's mixing-length theory

PRANDTL'S hypothesis^{1,2} about turbulent motion in a simple shear layer proposes that the typical values of the fluctuating velocity components in the x and y directions, u and v , are each proportional to $l\delta U/\delta y$ where l is the mixing length (*Mischungsweg*). Prandtl^{2,3} says that l "may be considered as the diameter of the masses of fluid moving as a whole in each individual case; or again, as the distance traversed by a mass of this type before it becomes blended in with neighbouring masses. . ."; and also that l is "somewhat similar, as regards effect, to the mean free path in the kinetic theory of gases". It follows that the Reynolds shear stress $-\rho_{uv}$ is proportional to

$$\rho l^2 (\delta U / \delta y)^2$$

and l is defined so that the constant of proportionality is unity. Prandtl, however, described this expression as "only a rough approximation".

Later work (ref. 4, and see ref. 5 for a summary) has shown that the formula for the mixing-length can be derived, using dimensional analysis, by assuming 'local equilibrium' between the turbulence and the mean flow. The only known example of a region of local equilibrium is the wall layer with $l = Ky$: experimentally $K \approx 0.41$. In a self-preserving (self-similar) flow with a single length scale δ , dimensional analysis gives $l/\delta = f(y/\delta)$. These results of dimensional analysis apply to very restricted cases (which were, however, used historically to test the mixing-length theory) and they have no relation to Prandtl's original derivation. In more general cases the quantity l defined by Prandtl has no simple connection with any meaningful scale of the mean flow or the turbulence.

Almost any method of flow visualisation contradicts Prandtl's idea of momentum exchange via lumps of fluid ('*Flüssigkeitsballen*'). Turbulence is an assembly of tangled vortex lines and sheets, dominated by the essentially three-dimensional phenomenon of vortex stretching. If a lump of fluid happens to move away from its former surroundings as an entity, it interacts strongly with those surroundings by the interconnecting vortex lines.

It seems that Prandtl could reconcile his idea with the results of flow visualisation because the predominant method of flow visualisation used in the early years^{2,6} of Prandtl's Institute at Göttingen, during the development of the mixing length theory, was the Ahlborn method—the observation of

particles sprinkled on the free surface of a water flow. Many papers and books in which appear photographs taken at Gottingen show that this method of visualisation is very effective in showing up vorticity normal to the free surface. Indeed, because the free surface acts as a plane of symmetry the vorticity vector is constrained to meet the free surface at right angles. The constraint clearly influences a given eddy to a depth below the surface of the order of the eddy size. It therefore affects all sizes of eddy and not merely the smallest, which contribute most to the vorticity. The three-dimensional character of the turbulence is destroyed near the free surface, leaving only two-dimensional circulations like those seen in, for example, Fig. 36 of ref. 7 (which are similar to the two-dimensional circulations of synoptic scale in the atmosphere, seen in satellite photographs of cloud patterns). The photographs of turbulent channel flow reproduced as Figs 39 and 40 of ref. 7 also show exaggerated two-dimensional circulations, quite unlike our modern concept of turbulent motion but amply justifying the name *Flüssigkeitsballen*: it must surely have been photographs like these that provided the inspiration for Prandtl's mixing-length theory of 1925. In 1934 Prandtl and Tietjens (ref. 7, art. 155) commented that "In the case of a three-dimensional fluid motion, it is necessary to study the flow not only on the surface but also in the interior of the fluid". It is, however clear from their inclusion of free-surface visualisations of turbulence that they did not fully appreciate the untypical nature of the motion in the plane of symmetry formed by the free surface.

It seems very likely that the mixing length concept is a good model for two-dimensional eddying motions like synoptic-scale atmospheric circulations: it should also be applicable to highly stable stratified flows in which vertical motion is suppressed.

These remarks are offered not as a criticism of Prandtl but rather to exonerate him from the criticism that the mixing length concept ignores the facts: it is an excellent description of the flow patterns seen on a free surface. Unfortunately, however, these patterns are not representative of turbulence in the interior of a fluid.

I thank Professors G. K. Batchelor and R. E. Luxton for their suggestions and discussions.

P. BRADSHAW

Department of Aeronautics,
Imperial College,
London SW7 2BY, UK.

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Field evidence relating to the origin of ~3,000 Myr gneisses in southern West Greenland

SOME prominence has been given recently to the results and implications of isotope age studies^{1–4} in the early Precambrian rocks of the Godthåbsfjord region, southern West Greenland. The studies were initiated after field investigations⁵ suggested that the Godthåbsfjord region may contain outcrops of the oldest rocks in the earth's crust. McGregor⁵ has grouped these rocks into an older and a younger set of gneisses (respectively the Amitsoq and Nûk gneisses) separated in time by dykes (the Ameralik dykes), meta-

volcanic and metasedimentary rocks (the Malene supra-crustals) and stratiform meta-anorthosites. Among conclusions drawn from the isotope work is that based on initial ratios of ⁸⁷Sr/⁸⁶Sr that the parents of the Nûk gneisses in the Godthåbsfjord area cannot have been derived by partial or complete melting of the older Amitsoq gneisses³. We wish to comment on the applicability of this conclusion to the Nûk gneisses mapped in the area immediately to the south.

A team from the University of Exeter, supported by the Geological Survey of Greenland and the Natural Environment Research Council, began work in 1972 on the Buksefjorden map sheet (63 V 1, Danish Geodetic Institute), which overlaps the south of the Godthåbsfjord region, (ref. 5, Fig. 17) and the summer of 1973 has seen the completion of 1:20,000 mapping of a coastal area about 15 km wide extending more than 60 km south from Ameralik to Sermilik^{6,7} (Fig. 1). Lithological units recognised by McGregor⁵ in the Godthåbsfjord region extend south of Buksefjorden and we agree broadly, but not in detail, with the interpretation of the sequence of events in the evolution of the Archaean rocks of West Greenland listed in the most recent publication concerning isotopic ages⁴. It is pertinent to point out that we cannot accept McGregor's terminology⁵ in its entirety.

Discordant, but intensely deformed, amphibolitic Ameralik dykes occur in leucocratic Amitsoq gneisses at granulite and high amphibolite facies on the northeast of Amitsoarsugssuaq and at the entrance to Alangordlia (Fig. 1). The Amitsoq gneisses and Malene amphibolites in Amitsoarsugssuaq are both injected and variably migmatized by granitic (s.l.) masses and gneisses which in the sense of McGregor are Nûk gneisses. The effect of this injection and migmatization on the Ameralik dykes is to bring about a progressive loss of coherence until the dykes become strongly attenuated wisps which locally have recrystallised to coarse hornblendites. The wisps ultimately become assimilated by the younger gneisses. The Amitsoq

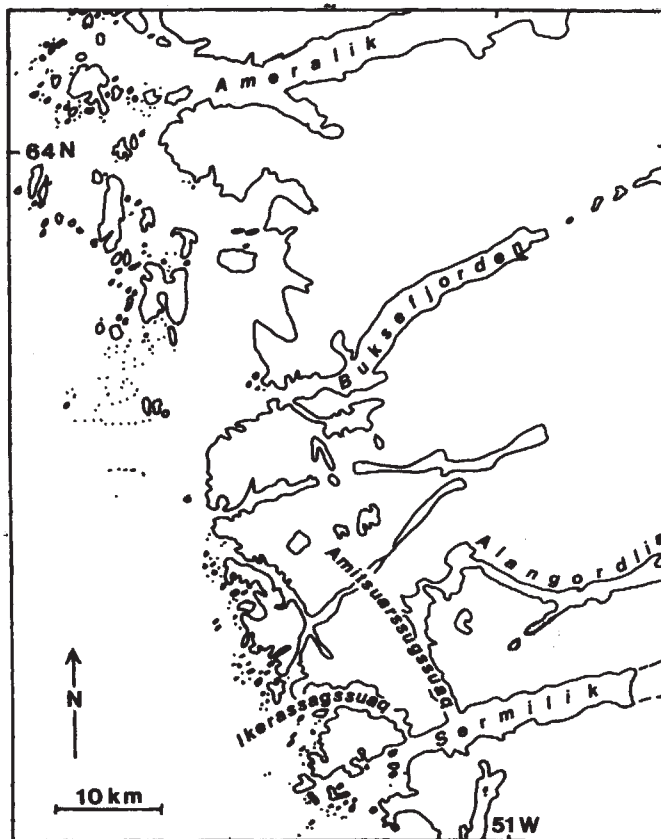


Fig. 1 Buksefjorden region of Greenland.

gneiss host to the dykes is also similarly reduced to xenolithic wisps and schlieren which merge completely into the younger gneiss. Similar effects are visible in coast outcrops around Ikerasagssuaq (Fig. 1). North of this inlet and around Amitsuarssugssuaq, the Amitsog gneisses, which for the most part are heavily migmatized with only rare Ameralik dykes, form linear strips within the Nûk gneisses, a relationship which suggests they have peeled off larger areas and are in an interrupted stage of incorporation into newer gneiss. Injected Nûk gneiss also occurs throughout the Buksefjorden region mapped so far. Original igneous textures survive only rarely: a hornblende granodiorite, for example, injected along a boundary between Amitsog gneiss and Malene amphibolite in east Amitsuarssugssuaq, retains igneous textures in the north but becomes gneissose with acid pygma in the south. Much of the Nûk gneiss elsewhere in the Buksefjorden region contains enclaves of Amitsog gneiss (some clearly xenolithic) in various states of preservation. Modifications of the younger gneiss and its inclusions to new, banded gneisses have taken place in association with straight belt formation, for example in Amitsuarssugssuaq. This we regard as synchronous with the granulite facies metamorphism which has given a $^{207}\text{Pb}/^{206}\text{Pb}$ isochron of $2,850 \pm 100$ Myr. (ref. 4).

The field evidence in the Buksefjorden region shows irrefutably that Nûk gneisses have been derived not only from injected material, but also by substantial remobilisation and incorporation of Amitsog gneiss. Our evidence is thus at variance with the conclusions drawn from isotope studies in the Godthåbsfjord region by Pankhurst *et al.*³, that the Nûk gneisses "cannot have been derived by partial or complete melting of older rocks similar to the Amitsog gneisses." It has also been pointed out⁴, that Sr and Pb isotope evidence suggests the $\sim 2,800$ Myr. granulite terrane in West Greenland does not represent a significant reworking of older crust (>100 – 200 Myr.) such as the $\sim 3,700$ Myr. old gneisses of the Godthåb area, and they conclude that a significant addition of crustal material from an upper mantle source must have taken place in West Greenland between $\sim 3,000$ – $2,800$ Myr. ago. The fact that Amitsog gneisses are common in the granulite facies terrane seen so far in Alangordlia and Sermilik (Fig. 1) does not in our view support this conclusion and we suggest, on the basis of field evidence for the late Archaean tectono-metamorphic evolution of the Buksefjorden region to be published elsewhere, that events associated with the granulite facies metamorphism and later intrusion of the Qorqût granite suite⁵ and related rocks may mark a stage as significant as the Nûk invasion postulated by McGregor in the addition of mantle-derived material to the Precambrian crust in West Greenland.

B. CHADWICK
K. COE
A. D. GIBBS
M. R. SHARPE
P. R. A. WELLS

Department of Geology,
University of Exeter

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Hardening of tungsten carbide by irradiation

WE report here a remarkable degree of hardening produced in standard tool-making grades of sintered tungsten carbide in a cobalt matrix by irradiation with charged particles.

Flat, rectangular blocks of three different grades (Wimet H, N and CXT) were bombarded with up to $5 \mu\text{A cm}^{-2}$ of 32-MeV ^3He ions in the Birmingham University 150-cm cyclotron. Each block was partially screened by thick aluminium plate which could be moved to expose increasing areas at intervals of 15 min. Each sample was thus divided into 10 separate areas which had been bombarded for periods of 0 to 135 min. The maximum dose given was therefore 1.6×10^{17} ^3He ions cm^{-2} or 3×10^{11} rad. The ions penetrated to a depth of about $140 \mu\text{m}$. The temperature of the block did not rise above 400°C during bombardment.

The hardness of each area after bombardment was measured using a Vickers Hardness testing machine, with a pyramidal diamond indenter, a load of 20 kg, and a 17-mm

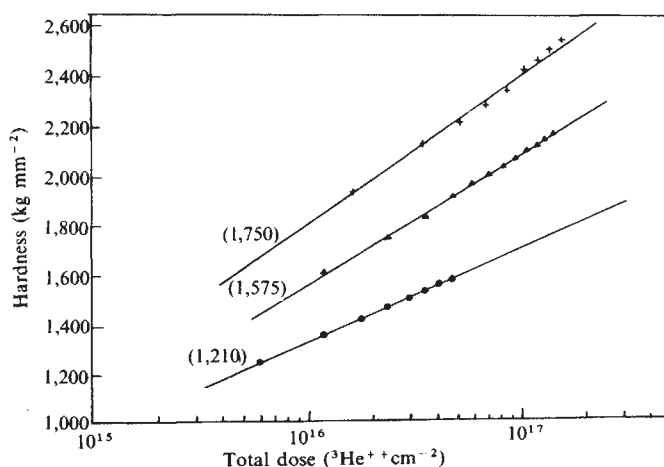


Fig. 1 Variation of Vickers hardness of three grades of Wickman Wimet tungsten carbide with bombardment of 32-MeV ^3He ions. The bracketed figures at the base of each line show the hardness of the unirradiated part of the sample. +, grade H; ▲, grade N; ●, grade CXT (Wickman Wimet grade).

objective lens for measurement of the indentation.

The results are shown in Fig. 1. In the range within which measurements were made the hardness is a logarithmic function of dose, and about 30% increase in hardness was given to the hardest material by a dose of the order of 10^{17} ions cm^{-2} . At this density some 50 or so ions will pass through each atom of tungsten or of cobalt in the surface of the block and extensive damage to the crystal matrix of either material can be expected.

Further details and a more extensive series of measurements, together with measurements of other properties of the irradiated material will be published elsewhere.

We thank Dr E. M. Trent for suggesting the measurement of hardness as an indication of the changes produced by bombardment and for permission to use the necessary equipment; and Wickman Wimet Ltd for providing the samples.

J. H. FREMLIN
N. A. ASKOURI

Department of Physics,
Chancellor Court,
The University,
PO Box 363,
Birmingham B15 2TT, UK

Received February 15, 1974.

BIOLOGICAL SCIENCES

Activation of maternal mRNA in the absence of poly(A) formation in fertilised sea urchin eggs

THE protein synthesis which follows fertilisation of sea urchin eggs depends largely on genetic templates synthesised much earlier during oogenesis¹. In the first 2 h after fertilisation the amount of protein being made increases steadily as the preformed mRNA molecules gradually become available for translation^{2,3}. During this period of mRNA activation, the polyadenylic acid content of the embryo increases to more than twice the level present in unfertilised eggs⁴⁻⁶. This fertilisation-stimulated polyadenylation occurs in the cytoplasm on RNA stored in the egg⁶ and is essentially complete by the time of the second cleavage. Soon after fertilisation the poly(A)-containing RNA moves from the post-ribosomal supernatant to the ribosomal-polysomal fraction of embryos^{5,6}, which suggests that polyadenylation is important in the activation of stored mRNA molecules. This is consistent with other reports that these tracts may play a role in the translation of viral RNAs^{7,8} and eukaryotic mRNAs^{9,10}. We have therefore investigated whether the formation of poly(A) after fertilisation is necessary for the activation of maternal mRNA. We measured the increase in protein synthesis and active mRNA while poly(A)

TABLE 1 Poly(A) assay of *Arbacia* eggs and embryos

RNA	ng poly(U) hybridised per μ g RNA	poly(A) (% total RNA)
Unfertilised eggs	0.357 $\pm$ 0.041 (8)	0.033
Four-cell embryos	0.739 $\pm$ 0.283 (7)	0.069
Four-cell embryos in 3'-deoxyadenosine	0.364 $\pm$ 0.054 (3)	0.034
Four-cell embryos in adenosine	0.725 (1)	0.068

The concentration of nucleosides was 1 mg ml⁻¹ artificial seawater. Cells were treated and poly(A) content assayed as in Fig. 1. The numbers in parentheses indicate the number of experiments performed.

synthesis was repressed and found a substantial activation even when poly(A) formation could not occur.

We assayed the poly(A) in *Arbacia punctulata* using the ³H-polyuridylic acid hybridisation technique of Gillespie *et al.*¹¹ and observed as reported for other species a doubling of poly(A) between fertilisation and 2 h, when the embryos are in the four-cell stage^{4,6}. Further increase was not observed for several hours. Poly(A) formation was inhibited by 3'-deoxyadenosine (Cordycepin), which blocks polyadenylation^{4,10,12} by terminating chain elongation. Sea urchin eggs were maintained in artificial seawater containing various concentrations of the adenosine analogue for 1 h, inseminated, allowed to develop in the continuous presence of the drug for 2 h, and assayed for poly(A) content. Increasing concentrations of the drug were found to inhibit the poly(A) formation progressively (Fig. 1), but extensive inhibition required relatively high concentrations. In seawater containing 200 μ g 3'-deoxyadenosine ml⁻¹, 67% of the postfertilisation polyadenylation was suppressed; 750 μ g ml⁻¹ prevented 96% of the increase in poly(A). Similar large doses of adenosine did not affect poly(A) titres (Table 1). Embryos allowed to continue growth in any concentration of 3'-deoxyadenosine up to 1 mg ml⁻¹, although delayed 30-60 min by late cleavage stages, did not arrest until blastula stage. Developmental arrest at this stage is expected since mRNAs transcribed during cleavage seem to be required for gastrulation and morphogenesis¹ and 3'-deoxyadenosine disrupts mRNA formation¹². It is surprising that such massive doses of the drug did not produce more general toxic effects.

Embryos in which the post-fertilisation addition of poly(A) to the maternal mRNA had been suppressed were tested for their ability to synthesise proteins. After 2 h of development in the inhibitor, they were incubated for

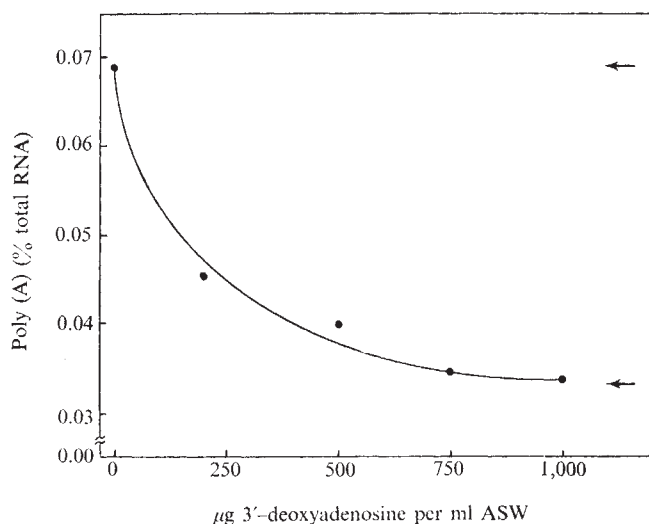


FIG. 1 Inhibition of polyadenylation by 3'-deoxyadenosine. Eggs were collected and washed by conventional procedures and maintained for 1 h on a rotary shaker at 21° C (ref. 2) in artificial seawater (ASW) containing 3'-deoxyadenosine (Cordycepin, Sigma) at the concentration indicated. The eggs were then inseminated with a 1:5,000 final dilution of sperm (95% fertilisation or greater was acceptable) and the embryos were allowed to develop for 2 h, at which time they were in the four-cell stage. Unfertilised eggs and embryos were washed once in cold acid seawater³, once in a 0.1M Tris-HCl buffer, pH 9.0, containing 15% sucrose, and homogenised in 10 volumes of 0.1M Tris-HCl, pH 9.0, made 0.5% with sodium dodecyl sulphate¹³. RNA was extracted from the homogenates with phenol and chloroform in a method essentially the same as that used by Slater *et al.*⁴ The ethanol-precipitated RNA, redissolved in hybridisation buffer, was washed twice with ether before being measured by $A_{260\text{ nm}}$. The poly(A)-content of the RNA was assayed according to the ³H-poly(U) hybridisation technique developed by Gillespie *et al.*¹¹ and applied successfully to sea urchin RNA by Slater, *et al.*⁴ Excellent results were obtained using 10 μ g sea urchin RNA per aliquot hybridised with 0.8 μ g of commercially prepared ³H-poly(U) (Schwarz/Mann), specific activity 23.6 Ci mol⁻¹. The upper and lower arrows indicate the poly(A) content of normal 2 h embryos and unfertilised eggs respectively.

TABLE 2 Protein synthesis in poly(A)-inhibited embryos and controls

Cells	Total incorporation (%)	Incorporation into nascent protein (%)
Unfertilised eggs	6.8 $\pm$ 0.03	1.7
Four-cell embryos	31.1 $\pm$ 2.9	8.6
Embryos in 3'-deoxyadenosine, 200 μ g ml ⁻¹	31.9 $\pm$ 1.3	9.1
Embryos in 3'-deoxyadenosine, 750 μ g ml ⁻¹	25.9 $\pm$ 2.8	7.4

Cultures of 3×10^5 embryos were treated with 3'-deoxyadenosine at the concentrations indicated as described for Fig. 1. Two hours after fertilisation, embryos were incubated for 5 min with 5 μ Ci ³H-leucine (30 Ci mmol⁻¹). Total soluble radioactivity and radioactivity incorporated into protein was determined by methods described previously². The standard deviation indicated is from three experiments in each case. Incorporation into nascent proteins was determined by preparing polysomes^{2,3} from embryos given ³H-leucine as just mentioned and dividing the total radioactivity in the polysome portion of the gradient by the total soluble radioactivity in the sample layered onto the sucrose gradient.

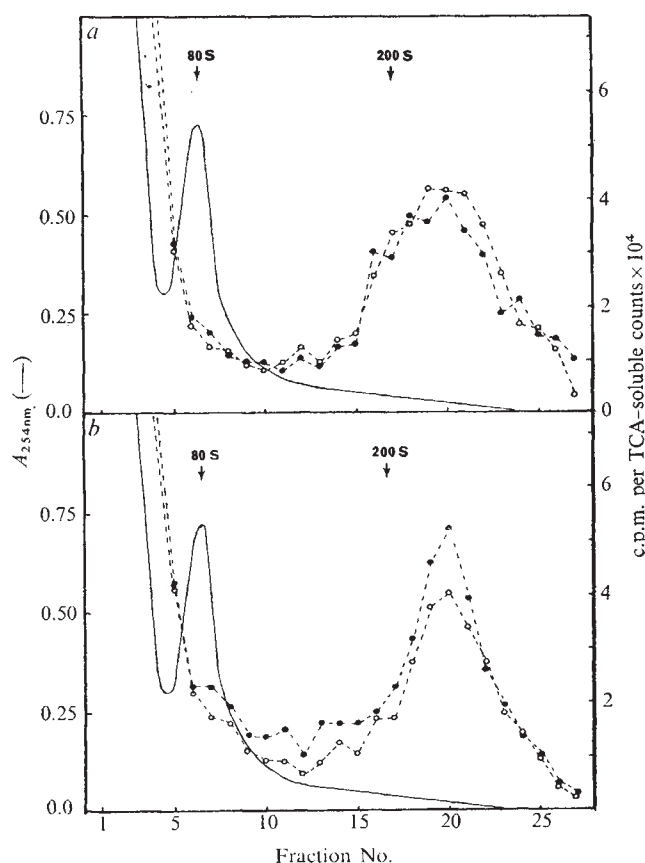


Fig. 2 Comparison of polysomes from normal embryos and embryos grown in 3'-deoxyadenosine at 200 $\mu\text{g ml}^{-1}$ (a) and 750 $\mu\text{g ml}^{-1}$ (b). A culture of 3×10^5 embryos was treated with the adenosine analogue as described in Fig. 1. At 2 h of development, the treated embryos and an equal number of control embryos were incubated for 5 min with 5 μCi ^3H -leucine (30 Ci mmol $^{-1}$). Polysomes were prepared from each culture with a high salt buffer as previously described^{2,3} and centrifuged on 30 ml, 10–35% sucrose gradients for 3 h at 22,000 r.p.m. in a Beckman SW25 rotor (5° C). ●, Control embryos; ○, treated embryos.

5 min with ^3H -leucine and the rate of protein synthesis was estimated as the percentage of the soluble radioactive pool which had been incorporated into trichloroacetic acid-precipitable protein. This estimate depends on the assumption that the inhibitor does not alter the size of the pool. As Table 2 shows, incorporation at 2 h of development was completely insensitive to 200 $\mu\text{g ml}^{-1}$ 3'-deoxyadenosine, which inhibits two-thirds of the normal poly(A) increase (Fig. 1). Increasing the level of drug to 750 $\mu\text{g ml}^{-1}$, to produce complete inhibition of the postfertilisation polyadenylation, had only a small effect on incorporation. It seems that protein synthesis can increase after fertilisation even without poly(A) formation.

The activation of protein synthesis when fertilisation takes place involves the translation of additional mRNA, which associates with ribosomes to increase the number of polysomes³. The ability of mRNA to enter the polysomes in sea urchins lacking the fertilisation-stimulated poly(A) sequences was investigated by preparing polysomes from the 3'-deoxyadenosine-treated embryos. Polysomes were measured as the percentage of the total soluble radioactive leucine incorporated into nascent proteins during a 5 min incubation with ^3H -leucine². There is no diminution in size or number of polysomes from embryos in the adenosine analogue at a concentration of 200 $\mu\text{g ml}^{-1}$, as compared with polysomes from untreated embryos (Fig. 2a). When the dosage was increased to 750 $\mu\text{g ml}^{-1}$ to eliminate all poly(A) formation, polysome size was not affected, although a slight

decrease in number of polysomes was apparent in embryos grown in this high level of inhibitor (Fig. 2b). The quantitative results of these measurements of nascent proteins on the polysomes (Table 2) confirm the relative insensitivity of the activation of maternal mRNA to suppression of polyadenylation.

Polysomes from 2 h embryos apparently normally contain mRNA which has been polyadenylated as a result of fertilisation^{5,6}. Our study suggests however that there is no direct correlation between the addition of these sequences and the ability of the mRNA to form polysomes. The finding that the postfertilisation synthesis of poly(A) is superfluous to the normal activation of maternal mRNA, coupled with the fact that it occurs in the cytoplasm^{4,6}, raises again the question of a role for poly(A) on mRNA. Evidence is accumulating^{5–8,14} that the result of polyadenylation may primarily be the conferral of a higher degree of stability to mRNA during its intracellular transport and translation. The findings presented here are not incompatible with the idea that maternal RNA templates which have been polyadenylated are more stable than those without poly(A).

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ANTHONY MESCHER*
TOM HUMPHREYS†

Marine Biological Laboratory,
Woods Hole, Massachusetts 02543

*Present address: Department of Zoology, Ohio State University, Columbus, Ohio 43210.

†Present address: Kewalo Marine Laboratory, University of Hawaii, Honolulu, Hawaii 96813.

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Triiodothyronine influences nuclear and extra-nuclear DNA in cultured human kidney cells

THE T-1 cell line, derived from normal human kidney epithelial cells and established over 15 yr ago by van der Veen *et al.*¹, has been found sensitive to and specific for the two thyroidal hormones, thyroxine (T_4) and triiodothyronine (T_3), as manifested by parameters reflecting morphology, proliferation, colony formation, and metabolism of lipids,

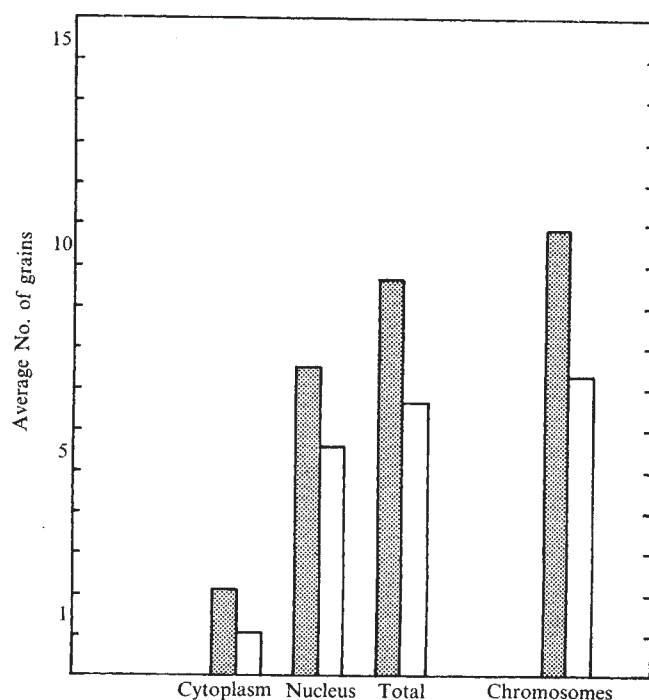


Fig. 1 Results of representative studies of ^3H -actinomycin D uptake by triiodothyronine (T_3) stimulated and control T-1 human kidney cells. Approximately 2×10^5 single cells were introduced into 100 mm Petri dishes (in duplicate) containing two microscope slides per dish. Cells stimulated for about 2 d with T_3 (8.90×10^{-7} M) as well as controls were labelled for 20 min with ^3H -actinomycin D (specific activity, 6.5 Ci mmol^{-1}) diluted to $0.5 \mu\text{Ci ml}^{-1}$, before rinsing with Hanks' balanced salt solution (BSS) and fixing with 50% ethanol-Hanks' BSS followed by absolute ethanol. Metaphase spreads of similarly treated and labelled cells were prepared by incubation with colchicine ($25 \mu\text{g ml}^{-1}$) for 16 h; hypotonic saline treatment and acetic alcohol (1:3) fixation completed the procedure. For autoradiography, cellular preparations and chromosome spreads were covered with Kodak NTB-2 emulsion, exposed for 14 and 11 d, respectively, followed by photographic processing and staining. Plotted are mean grain counts corrected for background, obtained by scoring 400 cells and 200 chromosome spreads; for each determination, the standard error of the mean was less than 5% of the indicated mean. $\square$, control; $\blacksquare$, T_3 stimulated.

proteins, and nucleic acids²⁻⁶. The cultured kidney cells therefore constitute a useful model for studying the peripheral actions elicited by these thyroidal hormones. Previous investigations strongly implicate the nucleus of the T-1 cell as being significantly involved in the response to T_4 and T_3 ; thus, thyroidal hormones affect nucleolar number, nucleolar RNA, and nuclear uptake of tagged T_4 (ref. 7). Here we report that T_3 acts on DNA throughout the T-1 cell in promoting the localisation of ^3H -actinomycin D, a labelled antibiotic that binds specifically to DNA^{8,9}.

For these determinations, autoradiographs were prepared of T-1 cells to evaluate ^3H -actinomycin D uptake by cytoplasm, nucleus, and chromosomes. Routinely, these cultured cells are grown as monolayers in Eagle's MEM supplemented with 10% foetal bovine serum, penicillin and streptomycin in 95% air-5% CO_2 incubators maintained at 37°C . For autoradiography, single cells in the exponential phase of growth, obtained by release and dispersal with trypsin, were overgrown onto microscope slides and, except for controls, stimulated for 2-3 d with T_3 (8.9×10^{-7} M) before tritium labelling; to similarly demonstrate the ^3H -actinomycin D associating with chromosomes, metaphase spreads of the labelled T-1 cells were made after incubation with colchicine for 5-16 h. After rinsing, fixing, and covering the slides with photographic emulsion, the exposed and processed autoradiographs were scored by counting developed grains. For

making background corrections, autoradiographs of unlabelled preparations were scored. In this manner, cellular ^3H uptake was determined (five independent experiments); also assessed was the number of grains associated with chromosomes (three separate experiments). Data derived from representative studies are plotted in Fig. 1.

Evidently, T_3 stimulation of the T-1 cells results in a marked augmentation in binding of tagged actinomycin D, especially to chromatin, as judged by the enhanced nuclear radioactivity of interphase cells and by the label found incorporated in the chromosomes at metaphase arrest. Also noteworthy is the rise in the extra-nuclear localisation of the tagged antibiotic effected by T_3 , possibly reflecting interaction between hormone and the mitochondrion¹⁰, a cytoplasmic organelle containing DNA and concerned with respiration and energy transfer.

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EDWARD SIEGEL
ELSIE P. SIEGEL

Departments of Radiology and Medicine,
University of Missouri Medical School,
Columbia, Missouri 65201

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Cytochalasin selectively inhibits synthesis of a secretory protein, cellulase, in *Achlya*

IN an effort to elucidate the possible relationship between cytoplasmic streaming and secretion we used the male strain E87 of the water mould *Achlya ambisexualis*¹. Cellulase secreted by *Achlya* presumably modifies the plasticity of the cellulosic walls of this fungus during morphogenesis². Synthesis of this enzyme can be induced by the steroid hormone antheridiol, by amino acids², or by shake induction (mechanical agitation) as reported here. In attempts to arrest streaming we used colchicine and vinblastine, which have been employed to disrupt microtubule function, and cytochalasins, whose reversible effects include inhibition of streaming³, of macromolecular secretion⁴, of uptake of small molecules such as glucose⁵, and destruction of microfilaments⁶.

Achlya mycelia were grown for 5 d in defined medium⁷ aerated by bubbling. After filtration, 1 g of mycelium was incubated in replicates in 10 ml of fresh growth medium lacking glucose. Glucose-free growth medium was used during shake induction, which is not dependent on exogenous glucose, to avoid the possibility that cytochalasin effects might be due to inhibition of glucose uptake. After 2 h incubation at room temperature on a reciprocating shaker at 120 cycles min^{-1} , 1 ml samples of medium and of aqueous mycelial extract were assayed viscometrically for cellulase using carboxymethyl cellulose as substrate⁸. The aliquot of mycelial

TABLE 1 Recovery of cellulase synthesis and secretion in *Achlya* after cytochalasin A (CA) treatment

Treatments	CA in medium*	Cellulase activity units	
		Medium†	Mycelium‡
1 h static preincubation	—	2.4	24.8
		2.6	21.8
1 h static preincubation	+	0.0	16.0
		0.0	16.1
1 h static preincubation, followed by	—, —	26.4	39.0
2 h shake induction in		24.4	39.4
fresh medium	+, +	0.9	14.2
		2.9	14.9
	+, —	12.2	20.5
		11.6	26.6

* Glucose-free growth medium, with 2% dimethylsulphoxide, without (—) or with (+) CA at 20 $\mu\text{g ml}^{-1}$.

† Values represent duplicate treatments.

‡ Mycelial cellulase levels at the start of preincubation were 14.8 and 15.1 units.

extract used for the assay represented 0.5 g fresh weight of mycelium. One unit of cellulase was defined as that amount which gave 1% reduction in the flow time of the enzyme-substrate mixture during a 1 h incubation at 37° C. Cytochalasins (Aldrich Chemical Co., Milwaukee, Wisconsin) were dissolved in dimethyl sulphoxide to give 1% (w/v) stock solutions.

In experiments with *Achlya*, a wide range of colchicine (1×10^{-4} M to 2.5×10^{-3} M) or vinblastine (5×10^{-5} M to 3.3×10^{-4} M) concentrations failed to inhibit streaming or cellulase secretion. Cytochalasin A (CA) inhibited both cellulase secretion and synthesis (Fig. 1). At 20 $\mu\text{g ml}^{-1}$ cytoplasmic streaming was rapidly and completely arrested. Cytochalasin B (CB) at this concentration failed to inhibit streaming, and its effect on cellulase synthesis ranged from mild inhibition to stimulation. These differential responses to cytochalasins are consistent with those of Betina *et al.*⁹, who reported that CA, but not CB, is fungistatic and morphogenically active on fungi.

The effects of CA are reversible. Streaming recovers within 30 min of transfer to inhibitor-free medium. Mycelium preincubated for 1 h in CA, then transferred to fresh medium for 2 h of shake induction, is capable of cellulase synthesis and secretion (Table 1). The CA-free controls demonstrate shake induction of cellulase. Mycelium maintained in CA shows little secretion of cellulase, and no increase in tissue

TABLE 2 Effect of cytochalasin A and cycloheximide on ¹⁴C-leucine incorporation into mycelial protein, and on final radioactivity of medium

Experiment	Treatment*	Total medium activity c.p.m. ml ⁻¹ after 4 h	Tissue protein c.p.m./100 μg after 4 h
I	Control	2209	3781
		2248	4552
	Cytochalasin A (20 $\mu\text{g ml}^{-1}$)	2785	4459
	Cycloheximide (1 $\mu\text{g ml}^{-1}$)	2644	5181
		2077	215
II	Control	2214	244
		312	174
	Cytochalasin A (20 $\mu\text{g ml}^{-1}$)	328	180
	Cycloheximide (1 $\mu\text{g ml}^{-1}$)	376	196
		398	299
		298	64
		306	60

* All treatments were in duplicate, and contained 1 g fresh weight of mycelium in 10 ml glucose-free growth medium containing 2% dimethylsulphoxide and 0.4 mM leucine. The specific activities of the DL-leucine-¹⁴C used in experiments I and II were 8.5×10^4 c.p.m. μmol^{-1} , and 7.9×10^3 c.p.m. μmol^{-1} respectively. The 4 h incubation included 1 h static treatment and 3 h of shaking. Radioactivity was determined using 15 ml of a solubilising liquid scintillation fluid¹³. Medium was sampled after incubation to determine total radioactivity. Mycelia were rinsed twice with 30 ml of unlabelled leucine medium, and ground with sand in 0.5 N NaOH containing 10 mM leucine. The homogenates were acidified with 2 drops concentrated HCl, stored overnight at 0°C, and centrifuged for 15 min at 5,000g. Pellets were washed twice with 2 ml 10% trichloroacetic acid containing 10 mM leucine, and resuspended in 0.5 N NaOH to a final volume of 2.1 ml. Aliquants were used for liquid scintillation counting, and for protein determination¹⁴. In *Achlya*, cycloheximide is a known inhibitor of protein, including cellulase, synthesis¹².

enzyme, when compared with the level at the start of preincubation.

The possibility that CA acts as a general protein synthesis inhibitor was excluded by the results in Table 2, which, in agreement with other reports^{3,10} show no cytochalasin inhibition of labelled leucine uptake from the medium, or incorporation into protein. Failure of CA to inhibit general protein synthesis in *Achlya* indicates that the selective inhibition of cellulase synthesis may be a secondary effect, resulting from inhibition of secretion.

Previous work using cytochalasins has shown a correlation between streaming and plant cell growth^{3,11}. Our results indicate the existence of coupling, not only between streaming and secretion, but between secretion and the synthesis of secretory protein, and raise the possibility that synthesis of certain proteins may be regulated at the level of secretion.

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DONOVAN DES S. THOMAS
MARJORIE LUTZAC
ELIAS MANAVATHU

Department of Biology,
University of Windsor,
Windsor, Ontario, Canada N9B 3P4

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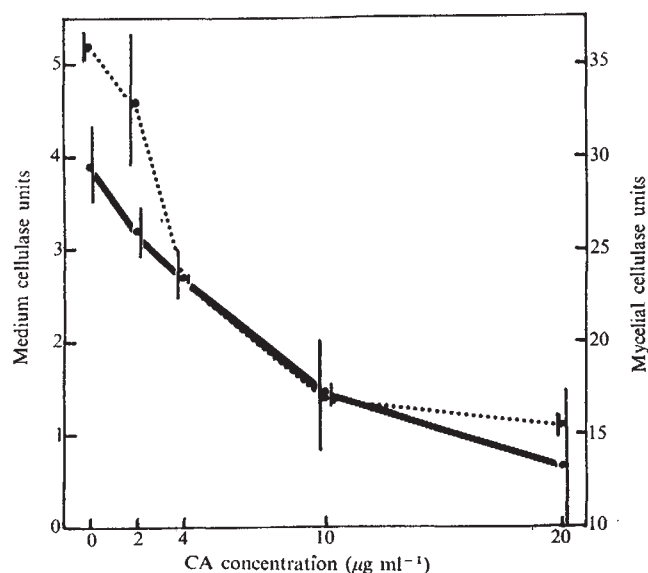


FIG. 1 Effect of cytochalasin A concentration on cellulase secretion and on mycelial cellulase. ●····●, Medium cellulase; ●——●, mycelial cellulase.

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Neurotransmitter synthesis in inner ear and lateral line sense organs

HAIR cells of inner ear sense organs and of lateral line organs in fish are innervated by afferent nerve fibres, to which they transmit information about mechanical stimuli. Efferent inhibitory nerve fibres, which originate in the central nervous system, also innervate most hair cells at their base (Fig. 1b). Recent evidence indicates that both these synapses are chemical; excitatory postsynaptic potentials (e.p.s.p.s) have been recorded in afferent nerve terminals in response to mechanical stimulation^{1,2} and inhibitory postsynaptic potentials (i.p.s.p.s) have been recorded in hair cells on stimulation of the efferent fibres². Furthermore, several findings suggest that acetylcholine is the efferent transmitter: i.p.s.p.s in hair cells, as well as the ensuing inhibition of afferent discharge, can be blocked by curare²⁻⁵; both acetylcholine esterase and choline acetyltransferase have been found in the efferent fibres⁶⁻⁹. The nature of the afferent transmitter however has not been elucidated, although catecholamines¹⁰ and glutamate¹¹ have been implicated.

We have investigated possible neurotransmitters in the inner ear and lateral line, especially at the afferent synapse. Three preparations were used: (1) the basilar papilla, an auditory organ in the bullfrog (*Rana catesbeiana*) which has only afferent innervation^{12,13} (Fig. 1a); (2) the lateral line canal organ of a teleost fish (toadfish, *Opsanus tau*) from which both the afferent and efferent innervations^{14,15} have been studied with intracellular recordings²; (3) the crista ampullaris of the semicircular canal in the skate (*Raja ocellata*), which has both afferent and efferent innervations and can be used for pharmacological studies.

We used a method based on the finding that cells which use a particular transmitter can synthesise and accumulate this transmitter in high concentrations¹⁶. Tissues were incubated in a medium containing radioactive precursors of noradrenaline, dopamine, γ -aminobutyric acid (GABA), acetylcholine (ACh) and serotonin. Tissue extracts were then fractionated by high-voltage electrophoresis and the radioactive products were identified^{17,18}. The activities of the enzymes involved in the synthesis of particular transmitters were also measured. Preliminary pharmacological experiments were also performed.

Three to eight organs from one of the three types described

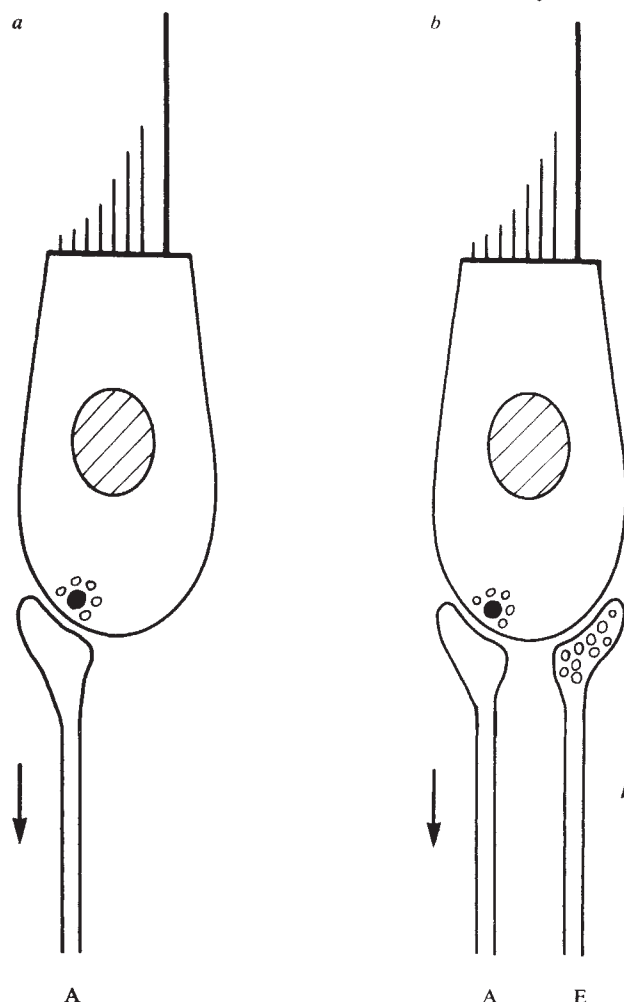


FIG. 1 Schematic diagram of hair cells and their innervation in (a) the frog basilar papilla, and (b) the lateral line canal organ and the semicircular canal ampulla.

above were dissected out and incubated at 20° C for 6–18 h in 30 μ l of medium (Leibovitz medium for frog (260 mOsmol) and toadfish (320 mOsmol), skate Ringer for skate) containing 100 μ Ci ml⁻¹ of one or a combination of electrophoretically purified L-³H-glutamic acid (generally labelled, 2.5 Ci mmol⁻¹), methyl-³H-choline chloride (6.7 Ci mmol⁻¹) and L-3,5-³H-tyrosine (2.5 Ci mmol⁻¹)^{17,18}. In two experiments, toadfish lateral line canal organs were incubated with L-methylene-¹⁴C-tryptophan (54.5 Ci mol⁻¹). After incubation, the tissue was first washed for 1 min in 2 ml Ringer and then homogenised with a ground glass homogeniser in 20 μ l 0.47 M formic acid–1.4 M acetic acid containing unlabelled precursors and transmitters. A 10 μ l sample of the homogenate and 10 μ l of the labelled medium

TABLE 1 Biosynthesis of possible neurotransmitters in inner ear and lateral line sense organs

Incubations	³ H-Noradrenaline	³ H-Dopamine	³ H-GABA	³ H-Acetylcholine
Frog basilar papillae	30 $\pm$ 26	32 $\pm$ 18	562 $\pm$ 85*	49 $\pm$ 15
Frog medium	32 $\pm$ 20	35 $\pm$ 20	74 $\pm$ 39	55 $\pm$ 20
Toadfish lateral line organs	30 $\pm$ 18	15 $\pm$ 9	2,350 $\pm$ 340*	556 $\pm$ 84*
Toadfish medium	28 $\pm$ 15	16 $\pm$ 10	122 $\pm$ 56	53 $\pm$ 27
Skate semicircular canal ampullae	33 $\pm$ 22	26 $\pm$ 16	1,252 $\pm$ 315*	145 $\pm$ 45*
Skate medium	30 $\pm$ 18	26 $\pm$ 16	65 $\pm$ 35	26 $\pm$ 16
Medium without incubation	29 $\pm$ 6	18 $\pm$ 8	56 $\pm$ 25	28 $\pm$ 15

The tissues were incubated in media containing ³H-tyrosine, ³H-glutamate and ³H-choline for 16 h. The numbers represent means $\pm$ s.d. of radioactive c.p.m. for 10 μ l homogenate or medium for three experiments.

* Value indicates that the synthesis in tissue is significantly different from background.

were used for high voltage paper electrophoresis (6,000 V, 1.5 h, pH 1.9)^{17,18}. After electrophoresis, the paper was dried, and ascending chromatography was applied perpendicular to the direction of electrophoresis to confirm the identities of the products (solvent for catecholamines and serotonin was: methyl ethyl ketone: propionic acid: water (200:65:55); solvent for GABA and acetylcholine was: *n*-butanol: acetic acid: water (60:15:25))¹⁹. After chromatography, the paper was dried and the spots corresponding to the precursors and transmitters were revealed by staining¹⁷. The regions corresponding to the appropriate transmitters and labelled precursors were cut into 1-cm squares and placed in scintillation vials each containing 1 ml 0.01 M HCl. After 2 h, 9 ml of Aquasol was added to each vial and the radioactivity was measured by scintillation counting.

In the basilar papilla and the lateral line canal organ the presence of glutamate decarboxylase (EC 4.1.1.15)²⁰ and choline acetyltransferase (EC 2.3.1.6)¹⁸ was also investigated. For measurement of glutamate decarboxylase activity, organs were homogenised in 40 μ l of L-¹⁴C-glutamate (4 μ Ci, uniformly labelled, 255 Ci mol⁻¹), 2-mercaptoethanol (10 mM), pyridoxal phosphate (1 mM), and KH₂PO₄ (200 mM, pH 7.3)²⁰. For determination of choline acetyltransferase activity, the organs were homogenised in 40 μ l of ³H-acetyl-coenzyme A (4 μ Ci, 980 Ci mol⁻¹), physostigmine (0.5 mM), KCl (100 mM) EDTA (0.2 mM, neutralised), choline (5 mM) and KH₂PO₄ (10 mM, pH 7.2)¹⁸. Each mixture was divided into four aliquots and incubated at 20° C for 0–8 h. The incubations were stopped by adding 1 μ l of 2M HCl to each aliquot. The reaction products were analysed by high voltage paper electrophoresis and the activities of labelled GABA and acetylcholine were measured by scintillation counting.

The experiments on transmitter biosynthesis indicated that the bullfrog basilar papilla with only afferent innervation synthesised GABA but no detectable acetylcholine, dopamine or noradrenaline (Table 1). On the other hand, the lateral line canal organ in the toadfish and the skate ampulla, which have both afferent and efferent innervation, synthesised GABA and acetylcholine but not dopamine, noradrenaline or serotonin. Although quantitative kinetics of enzymatic activities were not studied, our results indicated that the basilar papilla (Fig. 2a) contained glutamate decarboxylase but not choline acetyltransferase, whereas the lateral line canal organ contained both enzymes (Fig. 2b). These findings are consistent with the results of Table 1.

Thus, acetylcholine was synthesised in the lateral line canal organ and in the semicircular canal ampulla, both of which receive efferent nerve fibres, but not in the basilar papilla which lacks efferents. In addition, choline acetyltransferase was found only in organs with efferent innervation. This correlation strongly supports the notion that the efferent nerve fibres are cholinergic, as suggested earlier by physiological and biochemical^{2–9} results. The fact that curare, which specifically blocks cholinergic synapses, abolishes inhibition caused by the efferents but leaves afferent synaptic transmission unaffected, implies that a different transmitter is involved at the afferent synapse. Since GABA is synthesised by all three organs, all of which have afferent synapses that show the ultrastructural and physiological criteria for chemical transmission these results point to GABA as a putative afferent transmitter.

Our biochemical findings, however, do not exclude the possibility that the synthesis of GABA occurred in the afferent nerve fibres, non-neural elements or other unidentified neurones rather than in the hair cells. In an attempt to clarify this point, preliminary pharmacological experiments were performed on the isolated labyrinth of the skate²¹. In this preparation the horizontal crista ampullaris could be stimulated by hydraulic displacement of the cupula

and impulses from single afferent fibres could be recorded for several hours²². The base of the ampulla, where the nerve fibres enter, could be perfused with test solutions. Picrotoxin, a specific blocking agent for synapses transmitting with GABA^{16,23}, was found to block spontaneous as well as evoked discharge in sensory nerve fibres at moderate concentrations of the drug (5×10^{-5} to 10^{-6} M). This blockage was partly reversible in some cases. These experiments will be reported in more detail elsewhere.

Taken together, these findings give support for acetylcholine as the inhibitory transmitter used by efferent fibres of the acustico-lateralis system. In addition, our results raise the possibility that GABA is the neurotransmitter at the excitatory synapse between the hair cell and the afferent fibre. This possibility is interesting because in synapses where GABA has been shown to be the transmitter, its action on the postsynaptic cell is inhibitory^{16,24}. Thus, if future experiments substantiate our results, GABA would be an excitatory transmitter used by hair cells in the inner ear and lateral line sense organs.

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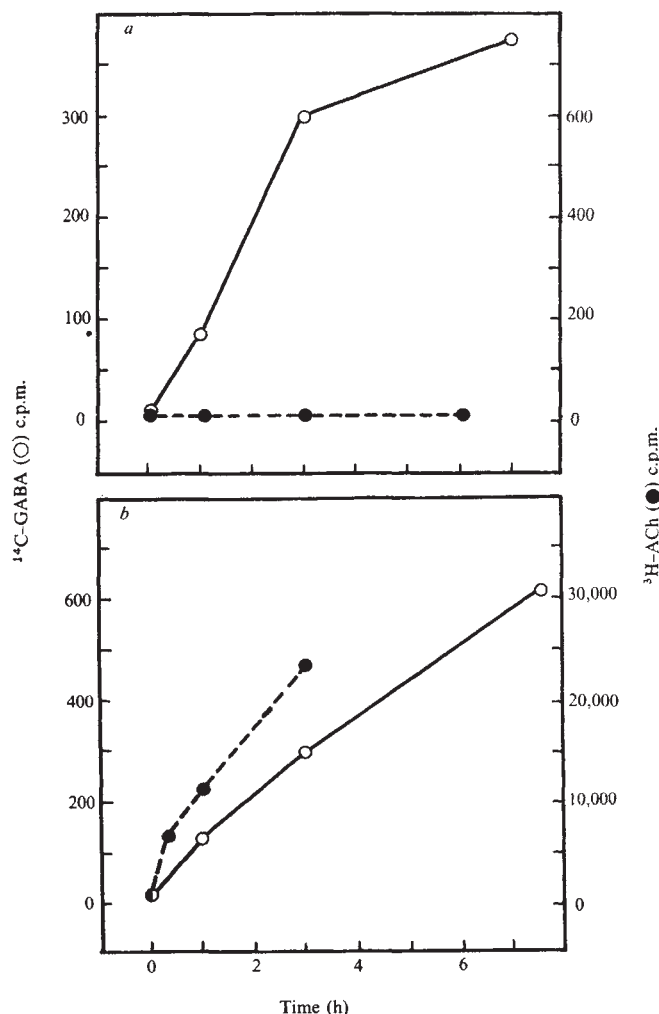


FIG. 2 Enzyme activities of glutamate decarboxylase (—) and choline acetyltransferase (---) in the bullfrog basilar papilla (a) and the toadfish lateral line canal organ (b).

recipient of a centennial award from the Medical Research Council of Canada.

ÅKE FLOCK

King Gustaf V Research Institute,
S-10401 Stockholm 60, Sweden

DOMINIC M. K. LAM

Department of Neurobiology,
Harvard Medical School,
Boston, Massachusetts 02115

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Release of specific protease during mitotic cycle of L5178Y murine leukaemic cells by sublethal autolysis

SUBLETHAL autolysis is postulated to be the process by which degradative enzymes may modify cell plasma membrane macromolecules without causing cell death^{1,2}. Malignant or virally transformed cells can be agglutinated by various agglutinins; their untransformed counterparts are not agglutinated, but normal cells become agglutinable upon mild treatment of the cells with a proteolytic enzyme³. Therefore, elevated levels of degradative enzymes may be important for maintenance of the malignant or transformed state through sublethal autolysis, which constantly modifies cell plasma membrane surfaces. This report shows that L5178Y cells release small amounts of a protease active at pH 7.8 and that release of this enzyme is almost exclusively in the M period of the L5178Y mitotic cycle. Rubin⁴ reported that small amounts of trypsin or Pronase produced an overgrowth phenomenon in cells; that is, contact-inhibited cells were released from contact inhibition and proceeded through a subsequent round of mitosis. Rubin⁴ has also isolated from cells infected with Rous sarcoma virus a similar non-dialysable, thermolabile overgrowth-stimulating factor.

The ability of lysosomal enzymes to modify the cell periphery has been documented and the control of cell growth through such modification has been reported⁴⁻⁶. Release of lysosomal enzymes during M phase in synchronous normal cells has been reported³, a proteolytic activity associated with

malignant cells mediating fibrinolysis has been said to be a general enzymatic change associated with transformation⁷, and increased concentrations of proteases have been found in a number of virally transformed cells compared with their normal counterparts^{1,8,9}.

L5178Y cells (mouse lymphoma cell line) were grown in suspension culture in sealed containers in Fischer's medium¹⁰ with 10% horse serum and were used in the exponential growth phase. Cell numbers were determined in a Coulter counter. Cells were synchronised by the method of Doida and Okada¹¹⁻¹⁴ by applying one treatment with excess thymidine followed by one treatment with colcemid and deoxycytidine. At each hour after release from the colcemid block 30 ml cell suspension were centrifuged out of solution at 1500g for 5 min, and the medium was assayed for proteolytic enzymes as given below. Simultaneously with the sampling for analysis, 3 ml cells were centrifuged out of solution, resuspended in 0.2 ml Fischer's medium containing 10 μ Ci of ³H-thymidine (15 Ci mmol⁻¹) and incubated at 37°C for 5 min. The incubation was terminated with 10% trichloroacetic acid; the cell suspension was centrifuged and the insoluble pellet washed twice with 10% trichloroacetic acid and once with ethanol-diethyl ether (2:1 (v/v)) dissolved in 1 M NaOH, plated on a glass filter disk, and counted in a liquid scintillation counter. Counts per minute from zero-time incubations, which were precipitated immediately, were subtracted from these results. This radioactive incorporation of ³H-thymidine is an indication of DNA synthesis in the cell and is a useful measure of synchrony and the S period. Protein was determined by the method of Lowry *et al.*¹⁵. Crystalline bovine serum albumin was used as a standard. Protein per cell increased linearly from about 0.9 mg per 10⁷ cells to about 1.7 mg per 10⁷ cells after about 9.5 h and then dropped back to 0.9 mg per 10⁷ cells. In general, there was a doubling of the cells at 9.5-10 h after release from the M block. Further details of these methods have been given previously¹²⁻¹⁴.

³H-Acetyl haemoglobin was prepared as described previously¹⁵⁻¹⁷. ³H-Acetic anhydride (400 Ci mol⁻¹) was reacted

TABLE 1 Influence of divalent cations and group-specific reagents on pH 7.8 proteinase of medium from exponential L5178Y cells

Addition	Final concentration (mM)	Relative activity (%) pH 7.8 proteinase
None	—	100
CoCl ₂	1.0	58
HgCl ₂	1.0	10
PbCl ₂	1.0	80
MnCl ₂	1.0	87
MgCl ₂	1.0	100
CaCl ₂	1.0	90
CdCl ₂	1.0	10
BaCl ₂	1.0	76
CuCl ₂	1.0	22
FeCl ₂	1.0	96
ZnCl ₂	1.0	6
EDTA	1.0	96
Diisopropylphosphorofluoridate	0.1	2
Cysteine	1.0	94
Mercaptoethanol	1.0	90
Sodium <i>p</i> -chloromercuribenzoate	1.0	24
2, 4'-dibromoacetophenone*	0.1	96
2-bromo-2-phenylacetophenone*	0.1	98
Iodoacetate	0.1	96
Ortho-iodosobenzoate	1.0	94

* Dissolved in dimethylsulphoxide diluted to 0.8% (v/v) in the assays.

The medium was incubated with the indicated compounds at the final concentration designated for 10 min at 25°C before the substrate was added. Data are given as relative activity; means from five independent determinations.

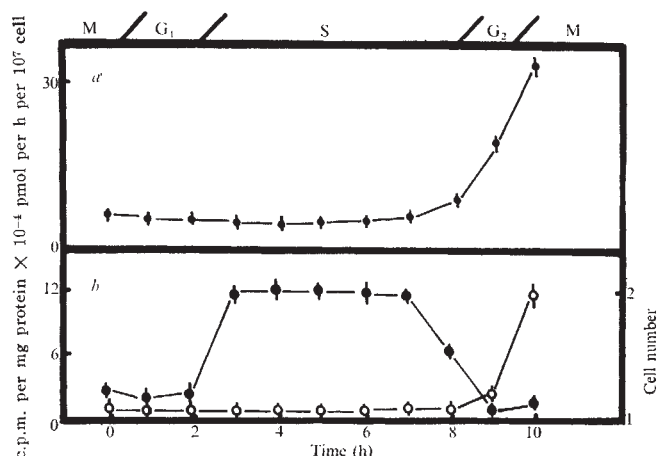


Fig. 1 Activity of released protease in synchronous L5178Y cells. Culture and synchrony of cells was as described previously¹¹⁻¹⁴. Experiments were performed by collective cells in the indicated growth phase, removing the cells by centrifugation at 1500g for 5 min and analysing the proteolytic activity at pH 7.8 of the medium as described in the text. Appropriate medium controls were assayed simultaneously, and the activity was subtracted from the total activity. Data are pmol of ³H-acetyl haemoglobin per 10⁷ cells and are means $\pm$ 1 s.d. from five independent observations. a, Released proteolytic activity. b, DNA synthesis during cell mitotic cycle as measured by incorporation of ³H-thymidine into acid-insoluble material (●) and cell number (Coulter counter; ○) in arbitrary units. A representation of the cell cycle is given above the panels¹¹⁻¹⁴. Cells were released from mitotic block just before zero time.

with the Type I beef blood haemoglobin. The material was purified to a specific activity of 162 c.p.m. pmol⁻¹ based on a molecular weight of 68,000 using the counting procedures described here. The specific activity of the material was 300 Ci mol⁻¹. Routinely in the assay systems described below, 50 μ l (136 μ g ³H-acetylated haemoglobin; 2 nmol; 324,000 c.p.m.; 0.6 μ Ci) of the ³H-acetylated haemoglobin was added per assay.

Routinely, 50 to 100 μ l of medium or lyophilised medium made up to a smaller volume were added to 100 μ l 0.1 M phosphate buffer, pH 7.8. As the substrate, 50 μ l of ³H-acetyl haemoglobin (2 nmol) were added. This mixture was incubated for 1 h at 37°C in a Dubnoff metabolic shaker. The reaction was terminated by placing the assay tubes in an ice-water bath (0-4°C) and adding 100 μ l of 2.5% haemoglobin and 50 μ l of 60% trichloroacetic acid (TCA). The precipitated material was removed by centrifugation at 5,000g for 5 min, and an aliquot of the supernatant fluid was plated on a glass fibre filter; the radioactivity was determined by counting in a liquid scintillation counter^{17,18}. Activity is expressed as pmol of haemoglobin degraded per h per 10⁷ cells. Suitable blanks consisting of medium without cells were added and incubated simultaneously.

Medium from exponentially growing L5178Y cells had 8 ± 1 pmol of haemoglobin per h per 10⁷ cells. Activity of 5'-nucleotidase, esterase, and UDPase (all enzymes present in large amounts in L5178Y cells¹²) were undetectable in the medium from the same cells, indicating that the proteolytic activity was probably actually released from the cells and that cell lysis was not occurring. The proteolytic enzyme in the medium was very dependent upon pH: although a slight peak of activity occurred between pH 3 and 4 (2 ± 0.6 pmol per h per 10⁷ cells), essentially no activity was present below pH 7.0 and above pH 8.6. Proteolytic enzymes can be classified in one of four groups: (1) serine proteinases, (2) acid proteinases, (3) thiol proteinases, and (4) metal activated proteinases¹³. As demonstrated in Table 1, the thiol-specific reagents, iodoacetate and *p*-chloromercuribenzoate, failed to substantially inhibit the proteinase (although the pH 7.8

proteinase was inhibited by *p*-chloromercuribenzoate); however, HgCl₂ was inhibitory to the enzyme. The data of Table 1 clearly show the released protease to be a serine proteinase.

The data of Fig. 1 show the enzyme to be most active in the M phase of the mitotic cycle; indeed during division at 10 h the enzyme reached peak activity of 30 ± 2 pmol haemoglobin per h per 10⁷ cells. The data clearly show that in these chemically synchronised cells a protease active at near physiological pH is exclusively released from the cell into the medium just subsequent to and during division. The role this enzyme plays in cellular regulation and control (if any) such as cell division, maintenance of the neoplastic state, overgrowth phenomena, sublethal autolysis, rounding up and lack of adhesion of cells during division, remains to be elucidated.

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H. BRUCE BOSMANN

Department of Pharmacology and Toxicology
University of Rochester School of Medicine and Dentistry,
Rochester, New York 14642

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Exencephaly in the syndrome of trisomy No. 12 of the foetal mouse

EXPERIMENTAL¹ and morphological² evidence suggests that exencephaly and anencephaly represent different degrees of severity of cranioschisis. Their morphogenesis seems to involve a primary disturbance of the cephalic somites and notochord. Both conditions are experimentally inducible in several species by the action of different causal factors and teratogens³⁻⁷. Moreover, exencephaly has been observed occasionally in gene mutants, for example in the mouse⁸, though rarely with complete penetrance in the affected strains⁹, and apparent autosomal recessive inheritance of neural tube defects has been suggested to occur at least in some instances in man^{10,11}. Therefore, it can be assumed that the aetiology of cranioschisis is at least complex and multifactorial involving environmental agents as well as

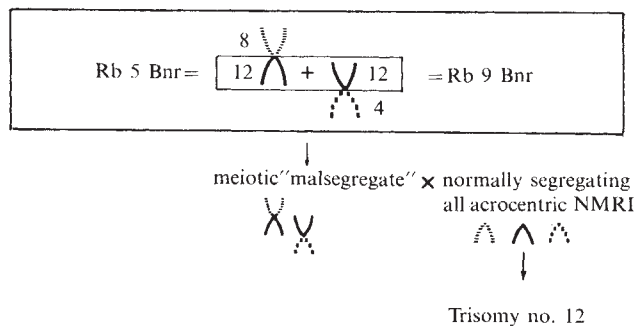


FIG. 1 The breeding of aneuploid zygotes by mating Rb5/Rb9 doubly heterozygous males with NMRI females.

genetic factors. But so far, defects belonging to this entity have not yet been observed in human or other mammalian chromosome mutation syndromes. In this respect it seems of interest to report the finding of exencephaly as constant and characteristic feature of trisomy of chromosome 12 in the mouse foetus.

Double structural heterozygotes possessing two metacentrics derived from Robertsonian changes and displaying monobrachial homology were used for inducing trisomy in the foetal offspring (A. G., D. Giers, and U. K., unpublished). Thus, for the study of trisomy No. 12, doubly heterozygous males with the Rb(8.12)5 and Rb(4.12)9 Bnr metacentrics (refs 12–14 and recommendations of subcommittee to consider rules for chromosome aberrations of the Committee on Standardized Genetic Nomenclature for Mice.) were selected and bred with NMRI (laboratory strain) females (Fig. 1). The two metacentrics share the chromosome arm which corresponds to mouse chromosome No. 12. In meiosis I they form a tetravalent (Fig. 2a) which is responsible for anaphase I malsegregation producing 43% chromosomally unbalanced, hypo- and hypermodal (Fig. 2b) spermatocytes II (A. G., D. Giers, and U. K., unpublished). On theoretical grounds it can be expected that among the aneuploid zygotes resulting from mating the Rb5/Rb9 double heterozygote with NMRI, the offspring with 41 chromosome arms in which both metacentrics had segregated together must correspond to the trisomy of the arm shared by them, that is of chromosome No. 12 (Fig. 1 and 2b).

Nineteen NMRI females mated with Rb5/Rb9 males were killed at day 10, 12, 13 to 14, and 19 (vaginal plugs controlled in the mornings; 'plug day' = day 1). Karyotypes were prepared from the total implant at day 10, and from the foetal membranes in the later stages of which the embryos were saved for morphological studies. At least 10 metaphases per implant were evaluated. At days 10 and

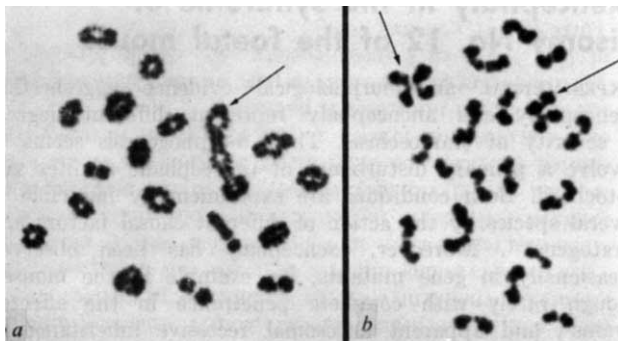


FIG. 2 a, Diakinesis (metaphase I) from Rb5/Rb9 male with tetravalent, b, Meiotic metaphase II with a hypermodal number of chromosome arms ($n = 21$) in the presence of the Rb5 and Rb9 metacentrics.

12 to 14, a total of 33 (21%) out of 156 living embryos were aneuploid (Table 1), of which 28 (28/33) showed 41 chromosome arms in the presence of the Rb5 and Rb9 metacentrics. Thus, they corresponded to trisomy No. 12. All of the 13 embryos of days 12 to 14 with the trisomy No. 12 karyotype which were morphologically studied exhibited fully developed exencephaly (Figs 3b and 4). The defect of the cranial vault is always complete and the brain protrudes beyond the cranium generally displaying a fairly good differentiation of the telencephalic hemispheres and of the diencephalic and rhombencephalic areas (Fig. 4). The eyes are remarkably small and hypoplastic. The size of most affected embryos is normal or only slightly subnormal. This is also true for the developmental stage characteristics, for instance development of the limbs and of the internal organs. In less than a fourth of the observed cases of cranioschisis, however, the development of the brain was more heavily affected, corresponding to more or less pronounced anencephaly (Fig. 3c). In addition these embryos were severely retarded and hypoplastic. At day 19, there were only three cases exhibiting exencephaly and

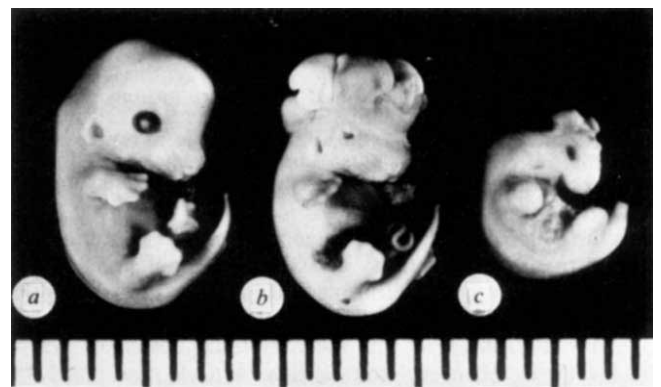


FIG. 3 Three embryos (on mm scale) of backcross progeny Rb5/Rb9Bnr male × NMRI female, day 13½; a, normal euploid littermate; b, trisomy No. 12 and exencephaly with microphthalmia; c, more severely affected and retarded trisomic embryo with anencephaly.

trisomy No. 12, but the affected embryos were already dead and autolysed. A more detailed histological study of trisomy No. 12 describing the changes of the brain, the skull basis, the eyes and other organs, for instance the placenta, will be the subject of a further report.

Two trisomic embryos (2/33 of days 10 to 14, one each of day 12 and 14) possessed only one metacentric (Table 1). It has to be assumed that in these cases the extra chromosome cannot be No. 12, but must be the result of meiotic malsegregation of a bivalent, though of undetermined origin. In fact, they were the only morphologically recognisable trisomies without cranioschisis. Their main external feature was marked hypoplasia. Moreover, at day 10, three triploid implants (3/33) were observed, all of them of maternal origin of the extra haploid set.

The present observations indicate that cranioschisis, and particularly exencephaly, is a constant feature of trisomy No. 12 of the mouse foetus. Exencephaly has never been encountered among the other autosomal trisomic conditions of the mouse so far studied in our laboratory (trisomy No. 1, 8, 10, 11, 13 and 15–17, A. G., D. Giers, and U. K. unpublished), or reported from other laboratories (trisomy No. 19, ref. 15). All these trisomic conditions cause death in the course of early or late foetal development, thus eventually leading to their elimination at least before term. In this respect, trisomy of chromosome No. 12 of the mouse can be well delineated and classified as displaying a characteristic

TABLE 1 Occurrence of aneuploidy and of macroscopically recognisable defects

	day 10		day 12		day 13/14		day 19	
No. of females	5		5		5		4	
No. of corpora lutea	65		73		65		57	
No. of resorptions	8		18		13		17	
Chromosome No.	2n =	40	41	40	41	40	41	40
No. of live normal embryos		35	—	46	—	42	—	33
No. of exencephalic embryos		—	18*	—	5†	—	8†	—
No. of otherwise abnormal embryos		—	—	—	1‡	—	1‡	—

* Morphology not determined. 15/18 2n = 41 with two metacentrics. 3/18 triploid.

† 2n = 41 in the presence of two metacentrics.

‡ 2n = 41 with only one metacentric. No exencephaly; embryos hypoplastic and retarded.

§ Freshly dead or autolysed.



FIG. 4 Front *a* and back *b* view (on mm scale) of exencephalic embryo with trisomy No. 12. Developmental stage day 13½ to day 14.

exencephaly or cranioschisis syndrome compatible with survival until a late period of gestation, possibly until day 17 or 18. The observation that its developmental profile surpasses at least the survival capacity of trisomy No. 1, 8, 11, 15 and 17 (A. G., D. Giers, and U. K., unpublished) lends support to the assumption that cranioschisis *per se* is not the primary cause of prenatal death in the trisomy No. 12 syndrome of the mouse.

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A. GROPP
U. KOLBUS

Institut für Pathologie
Medizinische Hochschule Lübeck
24 Lübeck, FRG

Received January 7, 1974.

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Phosphorylation of selected serine and threonine residues in myelin basic protein by endogenous and exogenous protein kinases

THERE is considerable interest in phosphoprotein metabolism in the brain and the effects of neurotransmitters on the turnover of phosphate in membrane proteins¹. Emphasis has been placed on the phosphorylation of proteins of synaptosomal membranes, but in addition myelin comprises a considerable proportion of the total substrate activity for a soluble protein kinase of brain². In the following paper Miyamoto *et al.*³ show that this kinase, which is stimulated by adenosine 3', 5'-cyclic monophosphate (cyclic AMP), phosphorylates the basic protein of myelin. Besides being a substrate for this soluble kinase the basic protein was found to be phosphorylated by an endogenous kinase of myelin⁴. Carnegie *et al.*⁴ demonstrated that a protein kinase from rabbit muscle would selectively phosphorylate certain serine and threonine residues in the basic proteins of rat and human myelin. Soluble protein kinases from brain and muscle seem to phosphorylate similar sites in histones⁵. We present evidence here that the endogenous protein kinase of myelin phosphorylates the basic protein, but not the proteolipid protein, and that the site of phosphorylation by this endogenous kinase is quite different from the site phosphorylated by soluble protein kinase.

Myelin was prepared from fresh bovine spinal cord by a rapid method⁶ and stored at -20° C. γ -³²P-ATP was prepared⁷ with a specific activity of 2500 d.p.m. pmol⁻¹. Protein kinase was prepared from rabbit muscle⁸ and detailed conditions for phosphorylation of proteins by this kinase are described by Kemp *et al.*⁹

Myelin (300 μ l, 7.5 mg protein) was incubated with 50 μ mol HEPES buffer⁹ pH 7.5; 29.3 nmol γ -³²P-ATP; 10 μ mol MgCl₂; 10 μ mol KF, in a final volume of 1 ml, for 10 min at 37° C. Samples (50 μ l) were removed and added to 1 ml of a solution of ATP (100 μ M) in 50% trichloroacetic acid. The precipitate was collected on glass fibre disks, washed repeatedly with trichloroacetic acid and ATP solution, and finally with ether.

Other incubations were performed as above but with the

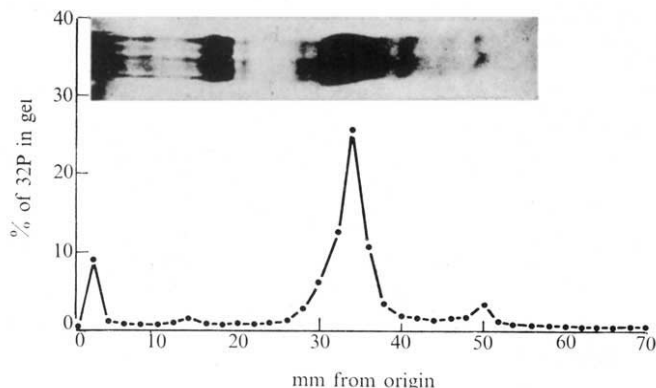


Fig. 1 Electrophoresis of myelin after phosphorylation by γ - ^{32}P -ATP and endogenous kinase. Lyophilised myelin (about 10^5 d.p.m.) was dissolved in phenol-formic acid-water (2:1:1, by volume) and separated by electrophoresis in the same solvent in a 15% polyacrylamide gel for 4 h at 550 V, 28 MA. Gels were stained with amido black and 2 mm slices were taken starting from the cathodic side of the slot which is not shown in the photograph of the stained gel. The slices were counted for ^{32}P in a flow counter with an efficiency of 30.8%. Proteolipid protein is the band at 17.5 mm and basic protein that at 34 mm.

addition of either 2 μM cyclic GMP; 2 μM cyclic AMP; or 100 μl of rabbit muscle protein kinase (5.28 mg ml^{-1}) plus 2 μM cyclic AMP. The total ^{32}P incorporated (d.p.m. per mg protein) into the myelin under these conditions were as follows: myelin alone, 3.0×10^5 ; myelin plus cyclic AMP, 2.8×10^5 ; myelin plus cyclic GMP, 3.0×10^5 ; and myelin plus cyclic AMP and rabbit muscle kinase, 4.4×10^5 . Thus cyclic AMP did not stimulate the endogenous kinase of bovine myelin. The addition of rabbit muscle kinase increased the total incorporation of ^{32}P into myelin. In other experiments, endogenous kinase did not appear to utilize γ - ^{32}P -GTP as a phosphoryl donor.

Phosphorylated myelin was collected from the incubation mixture by centrifugation and washed with 2×10 ml water before lyophilisation. Samples of lyophilised myelin from the above incubation mixtures were dissolved in phenol-formic acid-water (2:1:1, by vol) and subjected to electrophoresis in this solvent¹⁰ in a 15% polyacrylamide gel. Of the total radioactivity applied to gel 25% was not associated with any band of protein but was spread throughout the gel. After correction for this background 85% of the radioactivity was associated with the basic protein and none with the proteolipid protein (Fig. 1). Almost identical patterns, with no additional minor peaks or changes in the proportion (about 9%) of the protein of high molecular weight remaining at the origin, were obtained with myelin from the other incubation mixtures. In the sample with added kinase where the total incorporation of ^{32}P was increased, the pattern remained the same. Approximately 85% of the ^{32}P was incorporated into the basic protein.

Basic protein was prepared from each of the samples of lyophilised myelin by precipitation from chloroform-methanol¹¹. The basic protein was collected by filtration and the recovery of radioactivity was similar to that found by gel electrophoresis. Some difficulty was experienced in quantitatively eluting the phosphorylated basic protein from filter paper. That the phosphorylated protein was indeed the myelin basic protein was determined by several criteria. Amino acid analysis agreed with that of a sample of myelin basic protein obtained from bovine spinal cord. Gel electrophoresis showed only traces of proteins other than the myelin basic protein. The main peak of radioactivity coincided with the myelin basic protein and less than 15% was associated with the contaminants, with no individual contaminant containing more than 6%. The phosphorylated proteins were digested with trypsin¹² (treated with N-tosyl-L-phenylalanyl-

chloromethyl ketone) for 3 h at 37° C and peptide maps, stained with ninhydrin, were prepared as described in Fig. 2. These maps were identical with those from authentic basic protein.

From the specific activity of the phosphorylated protein approximately 25 mmol P were found to be incorporated per mol of basic protein. Autoradiography for 24 h was used to locate the ^{32}P -peptides. Analysis of the peptides was as described previously^{12,4} and phosphoserine was identified by electrophoresis¹³. Myelin basic protein from bovine spinal cord was phosphorylated and digested as previously described for the human and rat protein⁴.

The position of the main radioactive spots in a tryptic digest of ^{32}P -basic protein are shown in Fig. 2. The spots are labelled as described by Carnegie *et al.*⁴ for the phosphorylated human and rat proteins. Spot A, from these proteins, was not found in the digest of the bovine protein. Spots B, C and F were obtained in sufficient yield for amino acid analysis, which enabled their identification (Table 1). Edman degradation of the material in spot C showed that serine-110, rather than serine-112, was phosphorylated. Spot B was derived from peptide 6 and 7 in the bovine protein. It is well known that acidic residues adjacent to a basic amino acid inhibit tryptic digestion. Spot B was not conclusively identified in the human and rat proteins although it was shown to contain phosphothreonine⁴.

Spot G was not obtained in sufficient yield for accurate amino acid analysis. From a comparison of the effects of phosphorylation on the chromatographic and electrophoretic properties of peptides which were identified, however, it was possible to tentatively identify the parent peptides of spots G, K and O. The presence of these spots in digests of phosphorylated bovine, rat and human proteins suggested that they were derived from invariant regions of amino acid sequence⁴. Their low values of R_f and the presence of phosphoserine rather than phosphothreonine in hydrolysates of

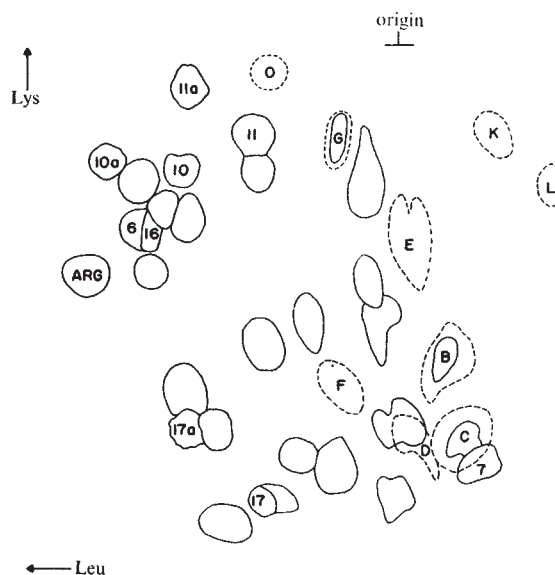


Fig. 2 Peptide map of phosphorylated bovine basic protein. The ^{32}P -protein was digested with trypsin and subjected to electrophoresis at pH 6.5 and chromatography in *n*-butanol-pyridine-acetic acid-water (15:10:3:12, by volume)¹². Peptides reacting with ninhydrin (—) are numbered as in ref. 15 and those labelled 'a' (for example, 10a Arg-Gly-Ser-Gly-Lys) are derived from the failure of trypsin to digest a bond at the N-terminal end of the peptide (for example, 10 Gly-Ser-Gly-Lys). Auto radiography for 24 h was used to detect the ^{32}P -peptides. The main spots (---) are shown and labelled as in ref. 4 as they had identical electrophoretic and chromatographic properties to the corresponding ^{32}P -peptides from phosphorylated human and rat proteins.

TABLE 1 Percentage distribution of radioactivity in peptides from a tryptic digest of ^{32}P -basic protein

^{32}P -Spot (see Fig. 2)	Derived from peptide No. (Fig. 2)	Amino acid sequence	^{32}P -Amino acid residue No. in protein	Incubation mixture with $\gamma^{32}\text{P}$ -ATP	
				Myelin + cyclic AMP	Myelin + RMPK + cyclic AMP
B	6 + 7*	His-Arg-Asp-Thr-Gly-Ile- Leu-Asp-Ser-Leu-Gly-Arg	Thr-34	1.1	1.7
C	17*	Gly-Leu-Ser-Leu-Ser-Arg	Ser-110	2.7	17.9
D	?	Unknown	Ser	0.9	4.0
E	?	Unknown	Ser	4.7	9.6
F	17a*	Gly-MMA-Gly-Leu-Ser-Leu- Ser-Arg	Ser-110	0.6	1.3
G	10a†	Arg-Gly-Ser-Gly-Lys	Ser-55	67.0	48.6
K	10†	Gly-Ser-Gly-Lys	Ser-55	5.4	3.4
O	11a†	Arg-Gly-Ser-Gly-Lys-Asp- Gly-His-His-Ala-Ala-Arg	Ser-55	8.2	6.7
Others	?	Unknown	Ser	9.4	6.8
					20.9
					42.8
					8.9
					8.4
					5.1
					4.7
					1.1
					0.8
					7.3

Incubations with myelin were carried out for 10 min whereas the incubation of isolated myelin basic protein plus rabbit muscle kinase was carried out for 180 min to enable a direct comparison to be made with the phosphorylation of the human and rat proteins⁴; incubation for 10 min gave a similar distribution.

* Identified by amino acid analysis, and ref. 4.

† Identified by chromatographic and electrophoretic properties (see text). MMA, monomethylarginine; RMPK, rabbit muscle protein kinase. Others, minor spots which had been 0.1 and 3% of the total radioactivity on the peptide map.

these spots eliminated a large number of peptides from consideration. From a comparison of the change in electrophoretic mobility caused by phosphorylation of peptides which were identified, it was possible to estimate the molecular weights of the parent peptides of spots G, K and O (compare ref. 14). After this comparison only two serine residues, 8 and 55, remained to be considered. It is known that trypsin gives rise to three peptides^{12,15} all containing serine-55 (Table 1) and these fit the calculated molecular weights of the parent peptides of G, K and O. Therefore, serine-55 was tentatively identified as a site of phosphorylation for the endogenous kinase especially as the sequence adjacent to it is invariant.

Phosphorylation of myelin basic protein by endogenous kinase occurs mainly at one serine residue, tentatively identified as 55 (Table 1). Radioactivity at this site represented 80% of the ^{32}P incorporated. When rabbit muscle protein kinase was incubated with myelin, phosphate was incorporated into serine residues 55 and 110. After allowance was made for the contribution of the endogenous kinase, it seemed that the rabbit muscle kinase was mainly phosphorylating serine-110. These results indicate that the two kinases have different specificities. The labelling pattern obtained with the endogenous kinase was unaffected by either cyclic AMP or cyclic GMP.

As indicated in Table 1, the longer term incubation of the isolated myelin basic protein with rabbit muscle kinase results in phosphorylation of both serine and threonine residues. Miyamoto *et al.*³ have reported that incubation of isolated myelin basic protein with the brain cyclic AMP-dependent kinase also leads to phosphorylation of serine and threonine residues. It will be important to determine whether the specificity of *in vivo* phosphorylation of myelin basic protein resembles that obtained with endogenous myelin kinase or with exogenously added kinases.

With the isolated bovine basic protein and rabbit muscle protein kinase the distribution of ^{32}P was similar to that obtained with the human and rat proteins except that a major spot A, His-Gly-Ser-Lys, was not obtained with the bovine protein as substrate. The sequence in the bovine protein in this region is -Gly-Arg-Ser-Lys- due to the deletion of His-Gly¹⁵. No major spot containing this serine was found which may indicate that close proximity of the arginine residue to the serine prevented its phosphorylation. Myelin basic proteins are heterogeneous as a result of methylation of arginine-107 (refs 12, 15). As was found with the human and rat proteins, the rabbit muscle protein kinase preferentially phosphorylated serine-110 in the unmethylated form of the bovine protein⁴. It should be emphasised that this site remains accessible when the protein is complexed with other proteins

and lipids of myelin. The accessibility of this region in myelin is supported by preliminary experiments which showed that arginine-107 could be readily methylated by methylase I (G. M. Jones and P. R. Carnegie, unpublished).

In summary, although there are 26 possible serine or threonines available for phosphorylation in the protein, only one of these (serine-55) is preferred by the endogenous kinase and one (serine-110) by the added kinase when the protein is complexed in myelin. Miyamoto *et al.*³ found the basic protein could be phosphorylated *in vivo* but the sites of phosphorylation have not been identified; it cannot be assumed that they will be the same as those demonstrated *in vitro*.

The observation that the myelin basic protein can be specifically phosphorylated opens up new approaches to the study of the possible physiological role of this protein in normal¹⁶, neurodegenerative¹⁷ and neoplastic¹⁸ conditions.

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P. R. CARNEGIE

P. R. DUNKLEY

The Russell Grimwade School of Biochemistry,
University of Melbourne,
Parkville, Victoria, 3052

B. E. KEMP

A. W. MURRAY

School of Biological Sciences,
Flinders University,
Bedford Park,
South Australia, 5042

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In vitro and in vivo phosphorylation of myelin basic protein by cerebral protein kinase

A class of cyclic AMP-dependent protein kinases may mediate a wide variety of physiological functions through phosphorylation of enzyme molecules or other cellular proteins¹. Certain hormones activate these kinases indirectly by causing the synthesis of cyclic AMP in particular organs. The myelin basic protein is a major protein of myelin in vertebrate brains and spinal cords. It has been purified to homogeneity and its amino acid sequence has been established^{2,3}. Myelin is a cell membrane substance as evidenced from its ontogenic origin. We wished to know if the basic protein serves as a substrate for a cyclic AMP-dependent protein kinase.

Protein kinase and myelin basic protein were purified from bovine brain according to the method of Miyamoto *et al.*⁴ and Eylar⁵, respectively.

Myelin basic protein was purified to homogeneity, shown by its migrating as a single band on acrylamide disc gel electrophoresis at pH 4.3 (ref. 6). The substrate activity of the myelin basic protein was compared with that of various proteins which are known to be substrates for the protein kinase. As shown in Table 1, ³²P was incorporated into myelin basic protein as rapid as into histone, when the proteins were incubated with protein kinase in the presence of ³²P-γ-ATP. Dependence of the reaction on cyclic AMP was also clear. Incorporation of phosphate into myelin basic protein was confirmed with gel electrophoresis followed by autoradiography.

The labelled myelin basic protein was hydrolysed in 6 N HCl at 100° C for 4 h to examine into which amino acid residue phosphate was incorporated. The hydrolysate was applied to high voltage paper electrophoresis, and labelled phosphoserine and phosphothreonine were detected. The ratio of phosphorylated serine and threonine residues was 7:9, whereas histone was phosphorylated only at a seryl

residue. The phosphorylation of myelin basic protein was inhibited by a modulator of cyclic AMP-dependent protein kinase, as was the phosphorylation of histone^{7,8}.

The phosphorylation of myelin basic protein was proportional to the time of incubation. After 24 h a plateau was reached, with 3.8 mol of phosphate incorporated into the protein molecule, indicating that the protein has four residues which accept phosphate. The extensively phosphorylated protein migrated more slowly, as was expected.

It seemed necessary to prove the *in vitro* phosphorylation of myelin basic protein under more physiological conditions. The myelin fraction was prepared from rat brains according to the method of Uyemura *et al.*⁹ and was incubated in the presence of ³²P-γ-ATP. Phosphorylation of myelin basic protein by endogenous protein kinase contained in the myelin fraction was demonstrated. Incorporation of phosphate was examined by hydrochloric acid extraction of the basic protein followed by disc gel electrophoresis. Autoradiography and determination of radioactivity in slices of the gel verified the phosphorylation of the basic protein.

We then performed an *in vivo* experiment. We injected 6.1 μCi ³²P-orthophosphate into the ventricle of rat brain by the method of Hayden *et al.*¹⁰ The brains were dissected 30 min after the injection and myelin basic protein was purified. Incorporation of labelled phosphate into the protein was demonstrated by autoradiography of disc gel electrophoresis.

The final proof was obtained by the demonstration of the presence of phosphoseryl and phosphothreonyl groups in native myelin basic protein from bovine brain. The contents of the two amino acid residues of the protein was determined by acid hydrolysis and electrophoretic separation of the phosphoamino acids followed by amino acid analysis. Each molecule of bovine myelin basic protein contains 0.07 mol of phosphoserine and 0.09 mol of phosphothreonine residues. The value was calibrated by a partial loss of the phosphoamino acids at acid hydrolysis.

So it is clear that myelin basic protein is present in the brain in a partially phosphorylated form, and we think that the protein is in a dynamic state between phosphorylated and dephosphorylated forms. Methylation and glucosylation of myelin basic protein have been already established. Until now myelin has been considered only as a stable component of neurones, but its function should be reconsidered.

In vitro phosphorylation of myelin basic protein was reported by Dr Carnegie after this study was completed at the 4th International Meeting of the International Society of Neurochemistry. Details of these experiments will be described elsewhere.

EISHICHI MIYAMOTO
SHIRO KAKIUCHI

Research Division and Clinical Laboratory,
Nakamiya Mental Hospital Hirakata,
Osaka, Japan

YASUO KAKIMOTO

Department of Neurology,
Institute of Higher Nervous Activity,
Osaka University Medical School, Fukushima-ku,
Osaka, Japan

Received September 10, 1973.

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TABLE 1 Comparison of phosphorylation of various substrates by cyclic AMP-dependent protein kinase from bovine brain.

Substrate	Amount (μg)	Protein kinase activity	
		Without cyclic AMP (pmol per 5 min per 4 μg enzyme)	With 1 μM cyclic AMP
Histone mixture	200	8.7	79.9
Lysine-rich histone	200	6.3	36.7
Slightly lysine-rich histone	200	4.1	31.7
Myelin basic protein	200	2.4	22.5
Arginine-rich histone	200	2.3	18.9
Casein	500	1.2	6.0
Protamine	500	2.4	4.3
Phosvitin	500	0.5	0.6
Bovine serum albumin	500	0.7	0.8

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The neurogenic and myogenic hypotheses in human (Duchenne) muscular dystrophy

X-LINKED recessive (Duchenne) muscular dystrophy (DMD) is the most common, consistent, and disastrous form of the diseases in man. Evidence that it is neurogenic¹ is open to criticism^{2,3}. Though an apparently similar condition in mice, often used as a model, may be neurogenic⁴, its different serology⁵ and mode of inheritance⁶ suggest a fundamental dissimilarity. The following observations on the human disease bring new evidence to bear on the issue.

In mammalian skeletal muscle, each fibre has near its mid-point a single endplate supplied by a single nerve fibre; more than one endplate is a rarity⁷⁻⁹. The axon of each lower motor neurone branches repeatedly to supply in this way very many muscle fibres¹⁰ often widely scattered through the muscle^{11,12}.

If DMD were neurogenic and mediated by the lower motor neurone, then in afflicted males the dystrophic X chromosome in every neurone should render all the musculature dystrophic, as in fact it is¹³. Likewise, in carriers of DMD, the normal early random inactivation as stable¹⁴ sex chromatin of one X chromosome in every female somatic cell¹⁵, including neurones¹⁶, would give an individually proportioned mosaic

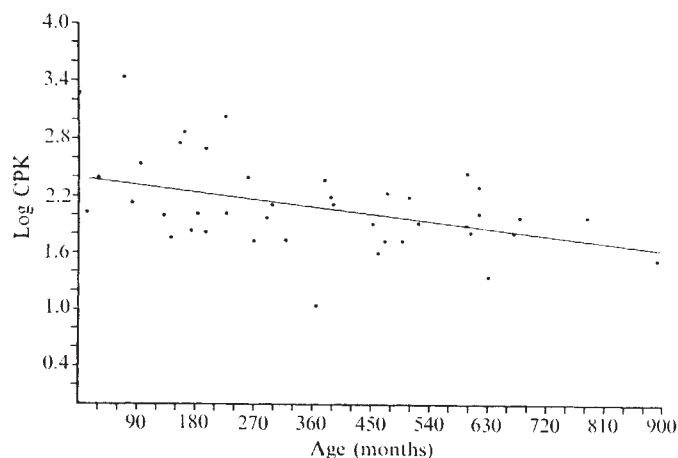


FIG. 2 Linear regression of log(CPK) on age in sera from female DMD carriers positively identified genetically and biochemically²¹. $n = 41$, $y = 2.398989 - 0.000848x$, $t = -2.87954$ on 39 d.f.; $P < 0.01$, $K_d = 0.001953$ per month, $t_{1/2} = 354.9886$ months (29.5824 yr).

of dystrophic motor neurones governed by the dystrophic X chromosome and healthy neurones by the normal X chromosome. Muscle fibres innervated by the former would then be wholly dystrophic, decaying as quickly as in afflicted males, but healthy if innervated by the latter. Thus carrier muscle should show a dual population of fibres, with clear-cut distinctions between them evident at biopsy. No such distinctions are seen, however. Instead, diffuse general changes are specifically noted¹⁷, and recent electron microscopic findings seem to show all carriers affected¹⁸. Nor, as we now show, is there biochemical evidence for the expected rapid decay of a population of dystrophic fibres.

In DMD, the easily-measured¹⁹ serum elevations of the muscle-specific enzyme creatine phosphokinase (CPK) due to the disease decline with age as the muscle mass diminishes, and covariance analysis of the data in Fig. 1 shows a constant process in which this exponential rate of decay of CPK is not significantly different between ambulant and wheelchair cases ($F = 0.0004$, 1 and 38 d.f.), with a joint estimate of slope of 0.00379 thus defining the disease. This slope gives estimated intercepts of 3.618 and 3.320 respectively, with a significant difference of about 0.3 ($F = 5.36$, 1 and 39 d.f., $P < 0.05$) implying on taking antilogs that CPK elevations are almost halved on transition from ambulation to the wheelchair. A third determinant is thus apparent, so that CPK in DMD specifically measures active dystrophic muscle mass, and may precisely assess prospective DMD therapy if activity and mass remain little changed over a short period.

In DMD the lifespan rarely exceeds 25 yr, but is apparently normal in carriers. Then if DMD were neurogenic, carriers should show initially a similar steep slope during decay of the dystrophic fibres, becoming horizontal later as in normal women¹⁹. Figure 2, however, shows a much gentler slope throughout, with covariance analysis giving no significant difference in the slopes before and after 30 yr. Its exponential rate of decline may be due to variable local anatomical disadvantages causing successive disappearance of a small number of fibres throughout life, occasionally evident at biopsy¹⁷. Its slightness is emphasised by consecutive CPK analyses over 3-7 yr in 22 carriers giving individual slopes neither significantly different from zero nor correlating significantly with initial age; clearly so small an interval with such a slope gives little information. In older carriers, moreover, derivation of probable earlier values from present CPK elevations gives reasonable results (obviously) if the

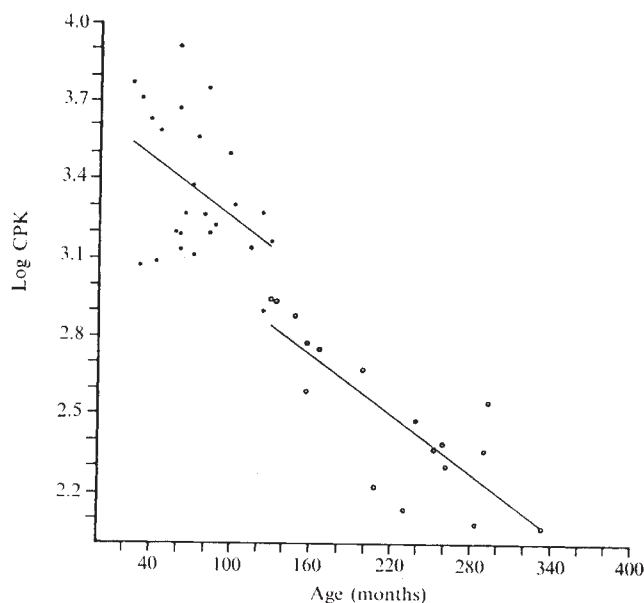


FIG. 1 Linear regressions of log(CPK) on age in sera from boys with clinically classical DMD, showing intercept and slope in equations providing exponential decay rate constant K_d and half-life $t_{1/2}$ of CPK values. Serum CPK throughout is assayed enzymatically¹⁹ and expressed in International Units as mU ml^{-1} at 25°C , with male and female normal upper limits of 58.1 and 37.7 mU ml^{-1} respectively. ●, DMD ambulant: $n = 25$; $y = 3.61886 - 0.003787x$, ($t = -2.22477$ on 33 d.f.; $P < 0.05$), $K_d = 0.00872$ per month, $t_{1/2} = 79.49036$ months (6.6242 yr); ○, DMD wheelchair: $n = 17$, $y = 3.318915 - 0.0037813x$ ($t = -5.70201$ on 15 d.f.; $P < 0.001$), $K_d = 0.008707$ per month, $t_{1/2} = 79.61014$ months (6.6342 yr).

slope for DMD carriers is used, but unacceptably high values if that for DMD ambulant is used, indicating that wholly dystrophic fibres cannot account for present persistent increases.

Further, the collateral reinnervation by other motor axon sprouts found in denervated muscle fibres²⁰ has now been described in the fragmenting fibres of DMD³, particularly in the evolving disease but not in the preclinical or terminal stages, with either minimal alteration or excessive damage. Then on the neurogenic view, if dystrophic carrier fibres decayed as quickly as in DMD itself, proportional reinnervation by adjacent normal axon sprouts and restoration towards normal of some dystrophic fibres should subtract them even more quickly, with a correspondingly more rapid decline of CPK than in DMD. Clearly this does not occur.

These findings and the wide range of sustained manifestation in DMD carriers, from undetectable to markedly abnormal²¹, are readily explained by random fusion during early growth of mononucleate myoblasts from clones with one or other X chromosome already inactivated, to give a dual population of nuclei, in different proportions for different individuals, in the mature multinucleated muscle cell¹⁷. In contrast, a neurogenic view based on orthodox carrier anatomy does not show how the commanding influence of a dystrophic lower motor neurone may be so extensively moderated by a healthy upper motor neurone and other connections, or *vice versa*, to give so wide a range of individually homogeneous carrier manifestation. Nor does it show how in DMD the invariable myocardial involvement, eventually lethal in patients and generally detectable even in carriers²², occurs without comparable innervation; no such involvement is seen²² in the putatively neurogenic²³ limb girdle type of muscular dystrophy. The myogenic view of DMD, however, does explain these observations, and if accepted in carriers applies equally to the disease itself in males.

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W. H. S. THOMSON
J. C. SWEETIN

Research Laboratory,
Knightswood Hospital,
Glasgow G13 2XG

R. A. ELTON

Medical Research Council Virology Unit,
Church Street,
Glasgow G11 5JR

Received January 17, 1974.

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Filaments and ribbons in vertebrate smooth muscle

SINCE Lowry *et al.*¹ found X-ray evidence for the presence of longitudinally ordered myosin in vertebrate smooth muscle there has been much debate on the precise form in which it is present. Small *et al.*^{2,3} have found well ordered and well preserved broad ribbon-shaped elements (80 Å wide and 200-1100 Å broad) in micrographs of vertebrate smooth muscle fixed after relaxation in isotonic and hypertonic salines. They believe that these ribbons are the myosin contractile elements and support this view with the observation that the meridional X-ray reflection of 143 Å (indicative of an ordered myosin structure) could arise from such ribbons.

Choi⁴, the first to observe thick filaments in vertebrate smooth muscles, found narrow ribbon-shaped structures in his preparations and so did Fawcett⁵. Many others, however, reported thick filaments in their preparations, either ribbon shaped, polygonal, or circular in cross section⁶⁻¹⁴.

Vertebrate smooth muscle myosin is very labile and its demonstration in fixed preparations depends on preparative procedures^{13,15}, therefore contradictory results were not unexpected and the importance of carrying out all X ray and electron microscopic work in conditions which correspond as nearly as possible to those *in vivo* must be emphasised. We think for the following reasons that work at low temperatures is unsatisfactory, and report studies on muscles relaxed at 37° C.

Lowry *et al.*¹ showed that the 143 Å reflection is best observed in muscles relaxed by cooling, and attributed the increase in intensity of the 143 Å reflection when the muscle is cooled as due only to suppression of movement of cross-bridges upon relaxation¹⁹ but at 0° C, changes in ionic content take place¹⁶ and in particular Ca ions are taken up by the tissue¹⁷ and the myosin appears to have undergone configurational changes¹⁸. Furthermore, we observe in the electron microscope that when *taenia coli* is equilibrated in ice-cold isotonic Bülbirg-Golenhofen saline¹⁸, as used by Lowry and also by Small, for different periods of time (30 min and 2, 4, 6 and 24 h) and the muscle is then fixed in 2.5% Taab glutaraldehyde buffered with 0.1 M cacodylate buffer at pH 7.0, the shape of the thick filaments varies with the period of equilibrium. After 30 min and 2, 4 or 6 h the thick filaments have mixed shapes, narrow ribbons, polygonal or circular as commonly seen with this method of fixation. After 24 h the thick filaments are mainly ribbon shaped (some quite broad ribbons can be observed—up to 770 Å wide) and some cell shrinkage has often occurred.

Like Small *et al.*^{2,3} we observe mostly ribbon-shaped thick filaments in *taenia coli* fixed with acrolein²⁰ after equilibrating the muscle for only 2 h in ice-cold saline before fixation, probably because acrolein penetrates more rapidly than glutaraldehyde (Fig. 1).

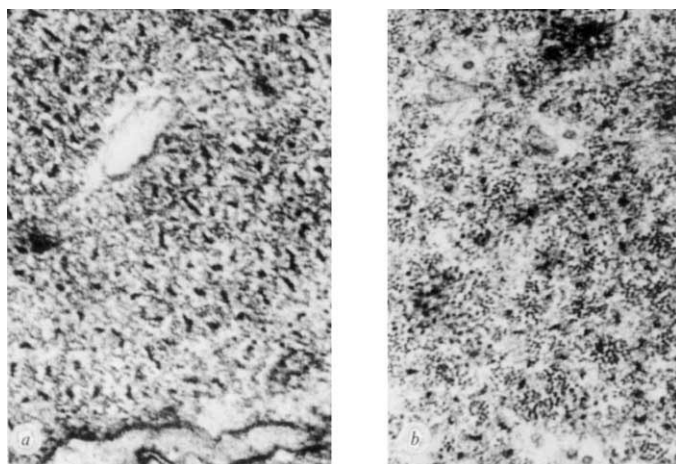


FIG. 1 *a*, Guinea pig taenia coli muscle equilibrated for 2 h in oxygenated Bülbiring-Golenhofen saline at 4° C. Primary fixation in acrolein²⁰. Sucrose was omitted from the fixative. Fixation was followed by an overnight wash in 0.1 M cacodylate buffer, pH 7.0, with additional 7% sucrose. Post fixation was in 1% osmium tetroxide dissolved in the washing solution. The muscle was under load throughout equilibration and fixation. Transverse section: note ribbons 80 Å by 320–560 Å ($\times 50,000$). *b*, Guinea pig taenia coli muscle equilibrated for 1 h in oxygenated Bülbiring-Golenhofen saline at 37° C. The muscle was then relaxed by the addition of xylocaine to the muscle bath (final concentration of xylocaine 1.5 mM). For the primary fixation 5% glutaraldehyde was added to the muscle bath. The muscle was under isometric tension and the tension was recorded throughout equilibration, the addition of xylocaine and primary fixation. Fixation was followed by an overnight wash in 0.1 M cacodylate buffer, pH 7.0, with additional 7% sucrose. Post fixation was in 1% osmium tetroxide dissolved in the washing solution. In this transverse section the thick filaments have ribbon shaped, polygonal and circular profiles. 80 Å by 160–320 Å ($\times 50,000$).

The size of the ribbons and the degree of shrinkage are very much enhanced if the muscles are equilibrated in cold hypertonic salines and indeed the 143 Å X-ray reflection is usually stronger and much clearer in a hypertonic solution at 4° C than it is in an isotonic solution (Fig. 2).

Also, if a muscle equilibrated for 24 h in the cold is transferred to an oxygenated isotonic saline at 37° C and the tension is recorded, it takes at least 30–40 min for the reappearance of activity and tension development, probably the time necessary for the ion concentration to return to normal and the ribbons to regain their normal shape.

We think therefore that the muscle undergoes considerable changes when it is cooled, and muscles should be studied at normal physiological temperatures (37° C).

Lowy *et al.*¹⁹ relaxed muscles at 37° C by removing calcium from the bathing solution, but such treatment does not make the 143 Å reflection stronger than that of a contracting muscle unless the bathing solution is also made hypertonic (a treatment which enhances the reflection in the cold). We decided to work on muscles equilibrated in an isotonic saline at 37° C and to relax the muscle with a drug if one could be found which did not affect the contractile mechanism of the muscle. The anaesthetic xylocaine (suggested to us by Dr Kiernan of the Health Sciences Centre, University of Western Ontario, Canada) proved satisfactory. When the drug was added to the organ bath in a concentration of approximately 1.5 mM the muscle lost all tension and activity, and showed no sign of activity or development of tension for periods lasting as long as 24 h. If after 24 h the xylocaine was washed away, the muscle recovered normal activity within minutes, suggesting that only the membrane had been affected by the drug. In addition, P. J.

Goodford and G. S. Wootton measured the wet weights and the extracellular space of muscles equilibrated in isotonic saline at 37° C with or without the addition of xylocaine and calculated from these measurements that although the cell volume decreased slowly in the control muscles, it stayed unchanged after a slight initial shrinkage in the xylocaine treated tissue. Therefore, in the xylocaine treated material, there is not likely to be an associated effect on the structure of the thick filaments which could affect the X-ray or electron microscope observations.

X-ray diffraction patterns were taken of taenia coli from adult guinea pigs equilibrated at 37° C in oxygenated Bülbiring-Golenhofen saline after the muscle had been relaxed by the addition of xylocaine. The X-ray patterns were taken with a mirror monochromator camera on an Elliott G $\times$ 13 X-ray generator; the tension of the muscle was monitored throughout. At 37° C the 143 Å reflection was not visible in xylocaine relaxed muscles although the actin equatorial reflections and the collagen reflections were good, so we should have seen the 143 Å reflection had it been present: one muscle was cooled to 4° C and the 143 Å reflection did then become visible.

Electron microscope observations were carried out on taenia coli from young guinea pigs (weighing less than 300 g), the muscles were treated in the same way as those used for X-ray observations and when they had relaxed completely after addition of the xylocaine they were fixed by addition of 2.5% Taab glutaraldehyde to the organ bath. The primary fixation which lasted for 1.5 h was carried out at 37° C, washing, post fixation and dehydration proceeded as previously described¹⁴. Thick filaments were present in many of the cells, but were not always numerous enough to account for all the myosin present in the cells. In cross section an assortment of narrow ribbons, polygonal and circular shapes were observed. (Fig. 1*B*). It has so far not been possible to observe a preponderance of narrow ribbons in cells from muscles after equilibration at 37° C.

Thus, in conditions in which the muscles can contract, when ionic and temperature conditions are appropriate for contraction we can find no X ray or electron microscopic evidence for the presence of broad ribbons.

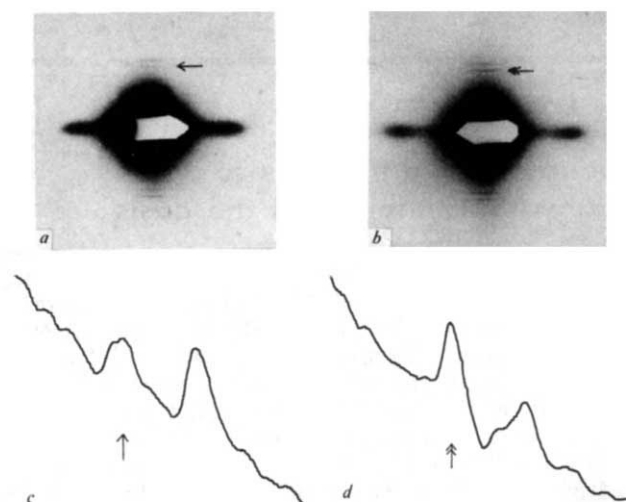


FIG. 2 *a*, X-ray diffraction pattern of a muscle in Bülbiring-Golenhofen saline at 5° C; *b*, densitometer trace of part of the meridian of pattern *a*, showing the 143 Å reflection (arrowed) weaker than the adjacent collagen reflection; *c*, X-ray diffraction pattern and *d*, densitometer trace of a muscle in a hypertonic (480 mOsmole) saline solution at 8° C. The 143 Å reflection (arrowed) is stronger than the adjacent collagen reflection. The actin equatorial reflection is much sharper and corresponds to a smaller separation of actin filaments than in the normal saline solution.

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CATHERINE F. SHOENBERG

Department of Anatomy,
Cambridge University,
Cambridge

JOHN C. HASELGROVE

MRC Laboratory of Molecular Biology, Hills Road,
Cambridge

Received January 7, 1974.

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Production of antibodies to tetrahydrocannabinol as the basis for its radioimmunoassay

TETRAHYDROCANNABINOL (THC) is the main pharmacologically active constituent of cannabis. A sensitive method for its measurement in plasma would be expected to have important clinical, experimental, and forensic uses. Brief descriptions of the production of antibodies to THC have appeared in the past¹⁻³. In one case², the antibodies were used to bind THC and its derivatives on a quantitative basis involving measurement of the inhibition of antibody fluorescence quenching or enhancement of hapten fluorescence when bound to antibody.

Here we use radioactively-labelled THC to detect the presence of antibodies. Each of six sheep was immunised with a different conjugate of THC and bovine serum albumin (BSA). Five milligrams of conjugate were dissolved in 2 ml of sterile water and emulsified with 4 ml of Freund's complete adjuvant. The mixture was injected intramuscularly into six sites in the medial surface of the thighs of a sheep. Thereafter animals were injected with 2.5 mg of conjugate

TABLE 1 Assay protocol

	100%	Zero	Test
Diluent buffer	0.5	0.4	0.4
Normal serum (1:2)	—	0.1	—
Antiserum (1:2)	—	—	0.1
³ H-THC (1 ng)	0.1	0.1	0.1
UCC (5%)	—	0.2	0.2

Values indicate volumes in ml. Sera were diluted with diluent buffer. ³H-THC was stored in dioxane and an aliquot diluted with diluent buffer before use. The incubation time was 1 h at 4°C. UCC was 5% charcoal in phosphate-buffered saline.

once a month and bled 7–10 d after each injection. One sheep produced an antiserum capable of binding ³H-THC and this material was investigated further. The conjugate which provoked antibody production was produced by preparation of the chlorocarbonate derivative of THC with phosgene and subsequent coupling of the derivative to BSA by the Schotten-Baumann reaction⁴. By the inclusion of a small amount of ³H-THC in the process, it was shown that an average of 12 haptens were conjugated to each BSA molecule.

³H-THC (2 μ Ci μ g⁻¹) was used to assess the binding capacity of the sheep antisera. One nanogram of labelled THC was incubated for 1 h in a mixture containing a 1:12 dilution of antiserum. The diluent buffer was 0.04 M phosphate, pH 7.4, containing 0.15 M sodium chloride and 0.2% bovine gammaglobulin. Separation of the free and antibody-bound fractions of the tracer was effected with 5% uncoated charcoal (UCC)⁵. Aliquots of the supernatant, containing antibody-bound tracer, were taken for β -counting (Table 1).

Table 2 shows the degree of binding of ³H-THC tracer exhibited by 1:12 dilutions of sera collected after successive booster immunisations. The results are expressed as the % of tracer present in the antibody-bound fraction (% bound). With serum taken following the third boost, the decrease in tracer binding produced by increasing amounts of unlabelled THC was measured. Figure 1 shows the calibration curve obtained with a 1:12 dilution of antiserum. The

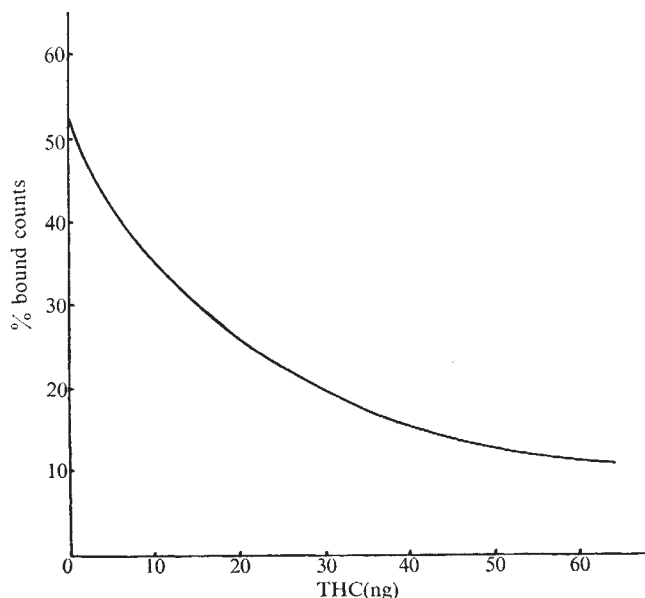


FIG. 1 Inhibition of tracer antibody binding by the addition of increasing amounts of THC to the assay system. The antiserum was used at a 1:12 final dilution and was that obtained following the third booster immunisation. Labelled and unlabelled THC were added simultaneously to the assay system and the incubation time was 1 h at 4°C.

TABLE 2 The ability to bind tracer of 1:12 final dilutions of the sera taken following monthly booster immunisations

Booster	% bound counts
1st	15.2
2nd	21.0
3rd	51.5

smallest amount of unlabelled THC producing measurable inhibition of ^3H -THC tracer binding was 2 ng. With higher specific activity tracer, it should be possible to lower the detection limit appreciably. Such a tracer would allow smaller amounts to be used in an incubation system containing less antiserum, and could constitute the basis for an immunological assay for THC with a sensitivity equal to or greater than those of physicochemical methods already in use⁶.

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J. D. TEALE
ELVYNE J. FORMAN
L. J. KING
V. MARKS

Department of Biochemistry,
University of Surrey,
Guildford, Surrey GU2 5XH

Received January 21, 1974.

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Structural analysis of human parathyroid hormone by a new microsequencing approach

RECENT evidence indicates that the synthesis of several polypeptide hormones, including parathyroid hormone, involves the production of precursor molecules larger than their corresponding hormones¹⁻⁵. The hormones are generated intracellularly by specific enzymatic cleavage. This process may represent an important control mechanism for hormonal synthesis, cellular storage and eventual secretion of the hormone. Thus complete structural characterisation of both precursor and product molecules for further peptide hormones in several species is clearly desirable. The precursor forms are often present in extremely low concentrations in glandular tissue, however, rendering difficult their isolation and sequence analysis by conventional chemical methods.

We describe here a microsequencing procedure based on automated Edman degradation of internally labelled polypeptides isolated in trace quantities from *in vitro* tissue incubation systems. We used this method, which is about 10,000 times more sensitive than the conventional technique for determination of amino acid sequences⁶, to confirm the structure of bovine parathyroid hormone⁷, and then to determine the structure of human parathyroid hormone.

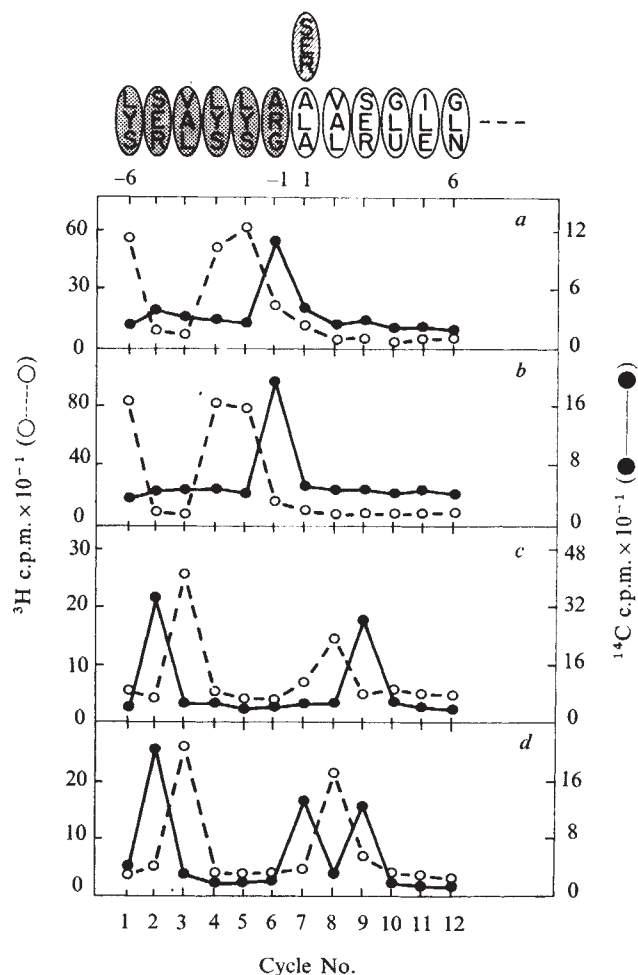
The bovine and human prohormones of parathyroid hormone were biosynthesised *in vitro* in tissue slices from bovine and human glands. During this process, radioactive lysine, serine, valine, and arginine were incorporated into the prohormone sequences (see Fig. 1 legend for details). These amino acids were chosen because they constitute the residues of the known bovine prohormone-specific hexapeptide. The prohormone fractions were isolated as described previously¹ and sequentially degraded in the protein sequenator.

The results of the sequence analyses are shown in Fig. 1. The sequence of the amino terminal portion of bovine prohormone is the same as that determined by conventional techniques⁷; human parathyroid hormone contains an amino terminal hexapeptide sequence identical to that of the bovine prohormone. At each cycle of the degradation where an incorporated radioactive residue was released from the prohormone, a clearly detectable, five to ten-fold increase in radioactivity above background was observed. The identity of each residue was confirmed by thin-layer chromatography of the radioactive amino acids removed at each degradation cycle. Approximately 85% of the radioactivity in each aliquot comigrated with the appropriate amino acid-phenylthiohydantoin standards.

We used two controls on the accuracy of the sequence analysis by the radioactive method. First, the analyses were continued for at least twelve cycles to overlap into the bovine and human parathyroid hormone structures already determined by conventional techniques⁸⁻¹¹, where the respective radioactive amino acids were detected in their proper positions (Fig. 1). Second, each cycle of the degradation was monitored for completeness of reaction by using gas-liquid chromatography¹² to measure the molar yield of each residue released from sperm whale apomyoglobin, which was used as a carrier protein in the sequence analyses. By this method each degradation could be shown to have a repetitive yield in the range of 92-94%¹³.

Since the additional amino acid residues present in the prohormone-specific sequence are not required for the expression of hormonal activity, one might expect, as with bovine and human proinsulin⁴, more amino acid mutations in the prohormone-specific peptide than in the active region or secreted form of the hormone itself. If, however, the parathyroid hormone-specific sequence, which is identical in human and bovine parathyroid hormones, is found in other species too, this may reflect constraints in sequence variation which are important for the efficient transformation of prohormone to hormone. Since the sequence of the biologically active region of human parathyroid hormone (first thirty-four residues) is known¹⁰⁻¹¹, a synthetic peptide¹⁴⁻¹⁵ can now be prepared that comprises the active portion of the human hormone with the additional prohormone-specific hexapeptide added to its amino terminal end. With this peptide, it should be possible to develop a radioimmunoassay for detection of the human prohormone, by techniques used previously with the bovine prohormone¹⁶. Such an assay should help to delineate the physiological importance of parathyroid hormone in man.

This highly sensitive microsequencing method represents the culmination of efforts in our laboratory and elsewhere to reduce significantly the quantity of protein or polypeptide needed for structural studies. Conventional non-radioactive Edman methods require at least 100 nmol of starting protein to accomplish a twenty-cycle degradation¹³. Our earlier use of a radioactive coupling reagent, ³⁵S-phenylisothiocyanate, for the automated Edman degradation, reduced the required quantity of protein to 5 nmol¹⁷. Recently we developed another microsequencing method, the degradation of radioiodinated proteins. The method is useful in determining the position of tyrosine and histidine residues in the sequence of polypeptides, available in only picomole quantities¹¹. With the degradation of internally labelled proteins described here,



sequence analysis involving all amino acids is possible with picomole quantities of polypeptide. Analyses reported here were carried out on 10 pmol of parathyroid hormone.

Certain issues concerning the use of a carrier protein to protect trace quantities of polypeptide from chemical and mechanical losses during automated Edman degradation have been clarified¹⁸. The use of such carriers to achieve protected degradation is essential for the success of micro-methods. In our earlier work polypeptides to be sequenced were not radioactive; rather, increased sensitivity was achieved with ³⁵S-phenylisothiocyanate. In this method, synthetic polypeptide carriers containing non-natural amino acids are necessary to avoid interference with the identification of the unknown sequence. In our new method internal labelling through biosynthesis enabled us to use as carrier a natural protein of known sequence; thereby conventional sequence analysis of the carrier protein provides a valuable monitor of the efficiency of each step of degradation of the trace, radiolabelled peptide.

Microsequencing methods involving radioactive amino acids promise to be generally applicable to the determination of the primary structure of trace quantities of other biologically important peptides. The work described here, as well as a study describing the sequence positions of a single radioactive residue (leucine) in a precursor to an immunoglobulin chain¹⁹, suggests that the internal labelling method can be expanded to include the use of all twenty natural amino acids as radioactive precursors in the *in vitro* biosynthesis of proteins of unknown sequence. Smithies *et al.* have used such an approach for amino terminal sequence analysis of immunoglobulin chains (personal communication). Where the approach through biosynthesis is not feasible, peptides could readily be labelled with tritium to high specific activi-

ties and sequenced²⁰. In some circumstances, sequence analysis of polypeptides bearing radioactive side chain prosthetic groups may be informative. These techniques that can be used to label polypeptides internally at high specific activity, coupled with the use of natural or synthetic carrier molecules, should extend the applicability of automated Edman degradation to sequence analyses of polypeptides of biological interest that are available only in trace quantities. Such advances, coupled with improvements in speed and efficiency of peptide synthesis²¹, represent an amplification essential to biochemical and physiological investigation of biologically interesting polypeptides since milligram or even gram amounts of pure polypeptides can be synthesised chemically, even though they cannot be obtained from natural sources.

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sequence information for human prohormone using similar methods²². Earlier, at the Keating symposium in September 1973, Cohn *et al.* presented preliminary data indicating that positions 1, 4 and 5 were likely to be lysine in the human prohormone structure.

J. W. JACOBS
B. KEMPER
H. D. NIALL
J. F. HABENER
J. T. POTTS, JUN.

Endocrine Unit,
Massachusetts General Hospital and
Harvard Medical School,
Boston, Massachusetts 02114

Received January 21, 1974.

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Effects of previous exposure on olfactory discrimination in *Acomys cahirinus*

EXPERIMENTS with chicks¹⁻³, rats^{4,5}, and rhesus monkeys⁶ indicate that previous exposure to a visual pattern facilitates subsequent discrimination learning involving that pattern and a novel visual stimulus. This facilitation of discrimination learning as a function of previous exposure has been found regardless of whether the exposure stimulus was the reinforced or the non-reinforced pattern in the subsequent discrimination task.

By comparison with animals exposed to only one of the

visual discriminanda before testing, those exposed to both stimuli generally take considerably more trials to master a discrimination task^{2,4}. Studies with monkeys and chicks indicate that animals that were familiar with both stimuli actually did less well on a discrimination task with those stimuli than did control animals for whom both of the stimuli were novel^{2,7}. For both hooded and albino rats, however, subjects familiar with both visual discriminanda learned a discrimination task faster than did control animals^{4,8,9}.

In general, such studies have consistently been concerned with visual stimuli; even though vision is not the dominant sense in the rat which is often used as a test species. Here we report a first attempt to investigate the effects of previous exposure to one or both discriminanda upon subsequent discrimination of olfactory stimuli. The subject species, *Acomys cahirinus* (spiny mouse) is a nocturnal murid rodent little used in laboratory experimentation.

Individual adult male spiny mice were randomly assigned to one of three treatment conditions ($n = 8$ per condition) which varied as a function of whether the animals were exposed to one, both, or neither of the olfactory stimuli for 7 d before the start of testing. The two olfactory stimuli, ethyl benzoate (EB) and acetophenone (AC) have similar diffusion rates and have been effectively used in previous studies with rats¹⁰, guinea pigs^{11,12}, and garter snakes¹³. The odourants were dropped onto cotton wool inside small plastic cannisters with holes drilled through the cap. Two of these cannisters were placed in the cage of each subject on the first day of exposure training and remained for 7 consecutive days. The cannisters in the cages of the control (C) animals remained empty throughout the training period; for the Experimental II (E-II) animals, one cannister contained AC and the second EB; the cannisters in the cages of the Experimental I (E-I) mice both contained the same odour (either AC or EB), which was randomly determined for each E-I subject. Mice were housed in four separate rooms as a function of the training odour(s) to which they were exposed.

Each animal was given a single 10 min habituation period in the olfactory discrimination apparatus (a T maze consisting of a runway with a short stem) on the first and second days of stimulus exposure. On days 3-7 of exposure, each mouse was given four pretraining trials during which he was placed into one end of the runway and allowed to run to the opposite end to obtain a small bit of apple. Discrimination trials began on day 8.

During discrimination testing, the two odours were blown into opposite ends of the runway, and two small food cups containing a piece of apple as a reward were placed into the runway—one at each end. The apple in the non-reinforced (incorrect) end was inaccessible behind a mesh screen. This procedure was adopted to equate for the presence of apple odour in each end of the runway. To obtain the apple reinforcement, the mouse had to approach the appropriate odour of the two being emitted into the T maze. A correction procedure was used¹⁴ whereby incorrect trials were repeated until the correct choice was made. Subjects were run for a total of four reinforced trials on each day until reaching a criterion of 8/9 correct consecutive responses—or until reaching a maximum of 50 reinforced trials. Throughout the training and discrimination testing, the apparatus was washed, rinsed and dried after testing each animal on each day.

For both the E-II and C conditions, four animals were reinforced for running to AC and four for running to EB. For each of the E-I animals, the designation of reinforced compared with non-reinforced odour was randomly determined. Thus, five E-I mice were reinforced for running towards the previous exposure odour, while for the remaining three E-I animals, the exposure odour was the negative (non-reinforced)

TABLE 1 Performance on olfactory discrimination test as a function of previous exposure to the test odour(s).

	Control (C) <i>n</i> = 8	Treatment condition	
		Experimental I (E-I) <i>n</i> = 8	Experimental II (E-II) <i>n</i> = 8
Mean reinforced trials to criterion	25.25	12.87	34.12
s. d.	11.22	11.04	10.27
		$H = 10.65, P < .01$	
Mean errors to criterion	15.9	5.0	21.5
s. d.	9.67	8.19	11.52
		$H = 10.305, P < .01$	

stimulus. The left/right positions of positive and negative odours was randomly determined each day for each subject, with the limitation that each odour would be introduced from each end for two trials per d.

Table 1 includes a summary of the results of the olfactory discrimination tests. Differences between the three conditions on the number of trials to criterion and errors to criterion were analysed with the Kruskal-Wallis test. As indicated in Table 1, significant differences ($P < .01$) were found between the treatment conditions on both dependent measures. Following the significant overall H tests, all possible paired comparisons for both dependent variables were tested using the Mann-Whitney U test. The results of these *post mortem* comparisons indicate that for both the number of reinforced trials to criterion and errors to criterion, the E-I condition had significantly lower scores than either the E-II or C conditions. When comparing trials to criterion for the E-I compared with C conditions, $U = 7, P = 0.006$ (2-tailed test) and for the E-I : E-II comparison, $U = 7, P = 0.006$. On the errors to criterion measure, the E-I : C comparison yielded $U = 7.5, P < .01$ and for the E-I : E-II comparison, $U = 5.5, P < .005$. No significant differences were obtained between the E-II compared with C conditions on either of the measures of discrimination learning (for trials to criterion, $U = 17, P = 0.13$; and for errors to criterion, $U = 22.5, P > 0.30$).

The behaviour of the E-I animals run to the exposure odour did not differ greatly from those for whom the exposure odour was the negative stimulus during discrimination testing; and both of these subgroups differed noticeably from the E-II and C conditions. The mean trials to criterion value was 15 for E-I animals for whom the exposure odour was the correct stimulus during testing, and 9.3 for those E-I animals for which the exposure odour was incorrect during subsequent testing. Mean errors to criterion values for these two subgroups of the E-I condition were 6.2 and 3 respectively.

These results indicate that spiny mice will learn to discriminate between a familiar and a novel odour faster than they will learn to discriminate between two unfamiliar odours. As animals in the E-I condition showed more rapid learning than did subjects of the other two conditions—regardless of whether the exposure odour was the rewarded or the unrewarded stimulus—this enhanced performance as a function of familiarity with one of the discriminanda cannot be due to previous attachment to the rewarded odour *per se*. These results tend to generally corroborate and extend those experiments in which previous exposure to one visual pattern has been found to facilitate subsequent discrimination between that pattern and a novel visual pattern^{1,4-6}.

The performance of the spiny mice exposed to one of the olfactory discriminanda (E-I) was superior to that of conspecifics who had been exposed to both discriminanda before discrimination testing. Those animals for whom both odours were familiar, performed roughly comparably to the

controls for whom both odours were novel. As we have mentioned however, earlier studies in which visual stimuli were used revealed that rhesus monkeys and chicks who were familiar with both discriminanda were inferior to controls^{7,2}, while albino and hooded rats, for whom both visual discriminanda were familiar, learned a visual discrimination faster than did controls^{4,8}. As pointed out by Chantrey², the visual stimuli used in these studies were probably more relevant to the diurnal rhesus monkeys and chicks than to nocturnal rodent species. On the other hand, olfaction (the sense under study here) seems to be the primary sense used by nocturnal rodents, including spiny mice. The relatively rapid acquisition of the olfactory discrimination test shown by the mice in the present study no doubt reflects the value of this capacity to nocturnal rodent species in coping with their environment. It may therefore be the case that as the value of any sense increases for the species in question, previous exposure to both discriminanda to be subsequently discriminated may tend to have little effect, or to interfere with acquisition—rather than facilitate subsequent discrimination between these stimuli. It must be emphasised, of course, that this is at best a very tentative *post hoc* hypothesis. A series of parametric studies using stimuli within several senses and with the same subject species would be required to test this hypothesis effectively.

Bateson and Chantrey^{7,2} interpret the poor performance of chicks and monkeys on a discrimination task in which both visual stimuli are familiar as being a result of the subjects classifying the stimuli together during the exposure period and subsequently having difficulty 'declassifying' them. In the present study, although the spiny mice that were exposed to both discriminanda did not differ significantly from the controls, they were inferior to those animals exposed to only one of the odours before testing (E-I). Therefore, it would seem that relative to the E-I condition, the E-II animals had trouble declassifying the two odours as a unitary stimulus complex as a function of their previous exposure; while the relatively poor performance of the control animals was a function of the total novelty of both discriminanda.

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RICHARD H. PORTER
JERRY T. TREADWAY

George Peabody College,
John F. Kennedy Center for Research on
Education and Human Development,
Nashville, Tennessee 37203

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Role of C3 in *in vitro* lymphocyte cooperation

COMPLEMENT is one of the best known effector systems of immunological reactions, and has also recently been shown to participate in non-immunological inflammation¹, and blood clotting²⁻⁴. Complement arises early in both phylogeny⁵ and ontogeny⁶, and the concept⁷ that it may in addition play a part in the induction of specific antibody production is of practical and theoretical interest. The complement C3-cleaving protein (CoF) of cobra venom and other C3-reactive agents have been shown in mice to inhibit the induction of thymus-dependent but not thymus-independent antibody responses *in vivo* (refs 7 and 8 and M.B.P., unpublished). Evidence was obtained which related suppression of antibody production to cleavage of C3, rather than to other possible effects of the C3-reactive agents, such as antigenic competition (refs 7 and 8 and M.B.P., unpublished). Interpretation of the data in terms of the role of C3 in the antibody response is difficult, however, especially as fluid phase C3 fragments (C3b and C3c) 'block' B lymphocyte C3 receptors and also suppress antigen-induced *in vitro* human lymphocyte transformation (refs 7 and 8 and M.B.P., and A. E. Butterworth, unpublished).

The requirement for C3 in cooperative antibody production was therefore studied *in vitro* where it is possible to exclude such effects as antigenic competition, and to differentiate between depletion of functional C3 and the generation of C3 fragments. The presence of murine C3 in the supernatant of mouse spleen cell cultures was first demonstrated and the effects on antibody responses of these cultures of isolated specific anti-mouse C3 antibodies were then examined.

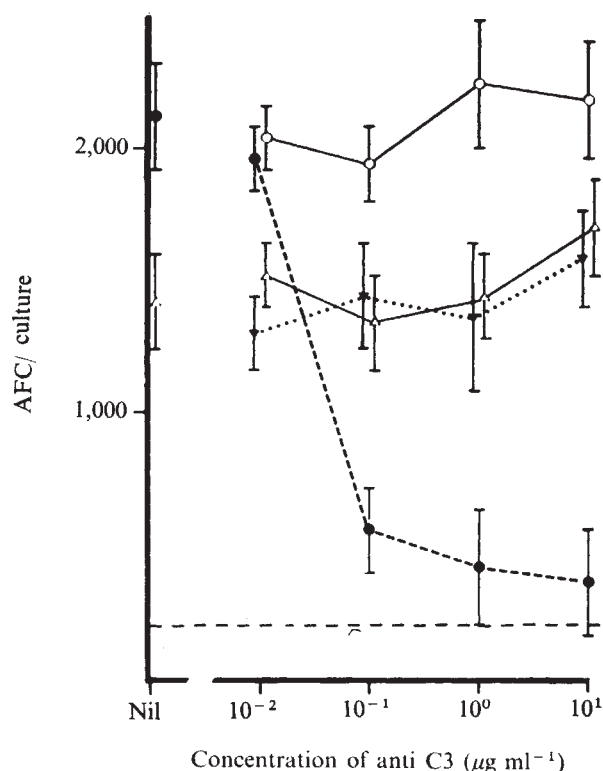


Fig. 1 Effect of anti-C3 on the response of TNP-KLH primed mouse spleen cells *in vitro*. ●, Response to TNP-KLH in the presence of anti-C3; ○, response to TNP-KLH in the presence of control IgG₁; ▼, response to DNP-POL in the presence of anti-C3; △, response to DNP-POL in the presence of control IgG₁; ----, background. Results from one seven experiments, using batch A. Values are arithmetic means of four cultures $\pm$ s.e.m. IgM response measured at day 4.

TABLE 1 Response to DNP-POL in serum-free medium.

Cells cultured	Antigen ($\mu\text{g ml}^{-1}$)	Antibody ($\mu\text{g ml}^{-1}$)	Anti-DNP response (AFC per culture $\pm$ s.e.)
Normal spleen*	Nil	Nil	55 $\pm$ 28†
Normal spleen*	DNP-POL ¹	Nil	612 $\pm$ 124
Normal spleen*	DPN-POL ¹	10 ¹ anti-C3	568 $\pm$ 86
Normal spleen*	DPN-POL ¹	10 ⁰ anti-C3	597 $\pm$ 104
Normal spleen*	DPN-POL ¹	10 ⁻¹ anti-C3	728 $\pm$ 131
Normal spleen*	DPN-POL ¹	10 ¹ IgG ₁	636 $\pm$ 154
Normal spleen*	DNP-POL ¹	10 ⁰ IgG ₁	532 $\pm$ 87

* 1.5×10^7 unprimed CBA spleen cells were cultured for 4 d in serum-free medium.

† Values are arithmetic means $\pm$ s.e. mean of four cultures per group.

CBA/H mice between 60 and 196-d-old, and bred at either University College, or at the Imperial Cancer Research Fund Laboratory, London, were used throughout. Some mice were injected intraperitoneally three times at 2 week intervals with 100 μg trinitrophenylated keyhole limpet haemocyanin (TNP-KLH) or KLH absorbed on bentonite⁹. They were used for culture 4 to 12 weeks later. TNP-KLH with 800 TNP groups per 8×10^6 daltons and dinitrophenylated-polymeric flagellin (DNP-POL) with 1.7 DNP groups per 40,000 daltons, prepared as described previously¹⁰ were used. Tissue culture was performed in Marbrook/Diener flasks^{11,12} using Eagle's minimal essential medium supplemented with sodium bicarbonate and 5% foetal calf serum (Biocult Laboratories, Glasgow). In some experiments, foetal calf serum was absent. Antibody-forming cells (AFC) were counted by the technique of Cunningham and Szenberg¹³. Anti-DNP AFC were assayed by using SRC coated with a dinitrophenylated rabbit anti-SRC Fab' fragment (DNP-Fab')¹⁴. Only the IgM response is reported here as the IgG response were relatively low.

Isolated anti-C3 antibodies were prepared from sheep anti-mouse C3 serum by elution from Sepharose C3 (ref. 8). The anti-mouse C3 serum was raised in sheep by a modification of the method of Mardiney and Müller-Eberhard^{8,15}. It was monospecific for C3, did not cross react with free or fixed bovine C3 from foetal calf serum, nor with either DNP-POL or TNP-KLH. The anti-C3 activity was estimated by agglutination of 1% sheep erythrocytes alexinated with mouse complement⁸; the protein concentrations and titres of the two preparations used in the present experiment were 90 $\mu\text{g ml}^{-1}$, 1/512 (Batch A) 170 $\mu\text{g ml}^{-1}$, 1/256 (Batch B).

Using a radioimmunoassay system (M. B. P., and D. V. Wilson, unpublished) to test for the presence of murine C3 in the supernatant of *in vitro* cultures of mouse spleen cells, it was found that the supernatants after 3.7 d culture of 1.5×10^7 spleen cells ml^{-1} regularly contained 0.3–0.6 $\mu\text{g ml}^{-1}$ of murine C3, assuming that normal mouse serum used as reference standard contains 1.0–2.0 ml C3 ml^{-1} .

The effect of isolated anti-mouse C3 antibodies on the thymus-dependent response (to TNP-KLH) and the thymus-independent response (to DNP-POL) of spleen cells from mice primed to TNP-KLH was investigated. Seven experiments, the results of one of which is shown in Fig. 1, gave the same result. Anti-mouse C3 suppressed the response to an optimal dose of TNP-KLH (10^{-2} $\mu\text{g ml}^{-1}$) but did not affect that to an optimal dose of DNP-POL (10^{-1} $\mu\text{g ml}^{-1}$). The IgG₁ fractions of the same anti-mouse C3 serum depleted of anti-C3 activity did not suppress the response to either antigen; nor did anti BSA antiserum at various concentrations, which would also induce the presence of immune complexes in the cultures (results not shown). The maximum suppression obtained in different experiments varied from 100% (response of background or less) to 70% or so. Preliminary studies indicate that the least suppression occurred with spleen cells taken from mice cultured

relatively soon after priming. Analogous experiments were performed with spleen cells from mice primed with carrier only, that is, KLH (Fig. 2). Again the response to TNP-KLH 10^{-1} $\mu\text{g ml}^{-1}$ was readily suppressed by anti-C3 while that to DNP-POL ($1 \mu\text{g ml}^{-1}$) was not.

The absence of suppression by anti-C3 of the response to DNP-POL was studied further, by using suboptimal concentrations of antigen. Responses to DNP-POL concentrations of 10^{-3} to $10 \mu\text{g ml}^{-1}$ were not affected by anti-C3 ($10 \mu\text{g ml}^{-1}$) (Fig. 3). It has been suggested that B cell activation *in vitro* may be dependent on bovine C3 provided by the foetal calf serum¹³. The sheep anti-mouse C3 which suppressed cooperative responses did not react with bovine C3, however, and unprimed spleen cells responded to DNP-POL in serum-free medium, even in the presence of anti-C3 (Table 1).

These results show that a thymus-dependent antibody response is suppressed by as little as $10^{-1} \mu\text{g ml}^{-1}$ of isolated anti-mouse C3 antibodies, a concentration comparable with that of murine C3 present in the culture supernates. In contrast, the thymus-independent response to DNP-POL was unaffected by as much as $10 \mu\text{g ml}^{-1}$ of anti-C3 even when suboptimal doses of antigen (Fig. 3) or serum-free cultures (Table 1) were used. Cooperative antibody production, such as induced by TNP-KLH, which requires the participation of three cell types namely T and B lymphocytes and macrophages^{17,18} thus requires C3, whereas no such requirement was demonstrable in a non-cooperative response induced by DNP-POL which only involves B cells¹⁹. Both B cells²⁰ and macrophages²¹ have surface receptors, albeit of different types, for fixed C3, and it is therefore possible that the role of C3 in lymphocyte cooperation may be expressed at either the B cell level, the macrophage level

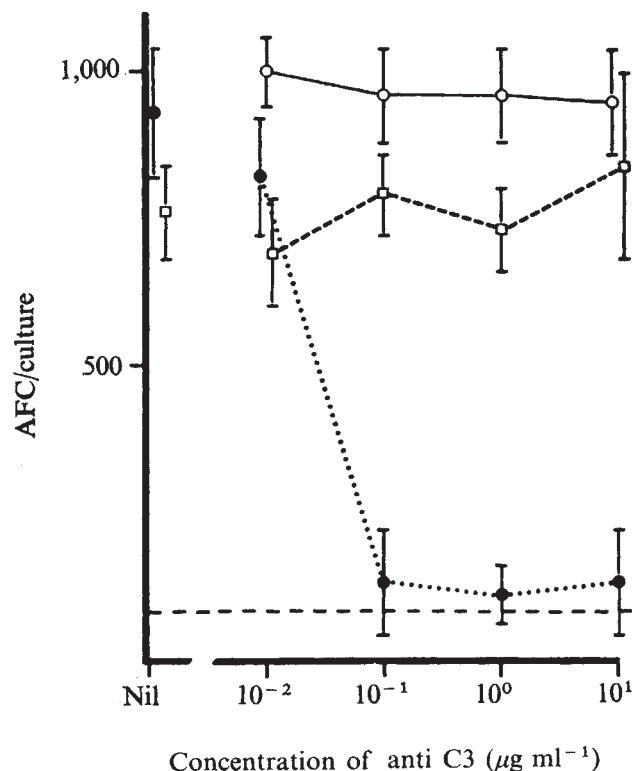


Fig. 2 Effect of anti-C3 on the response of KLH-primed mouse spleen cells *in vitro*. ●, Response to TNP-KLH in the presence of anti-C3; ○, response to TNP-KLH in the presence of control IgG; □, response to DNP-POL in the presence of anti-C3; ----, background. Results taken from one of six similar experiments using batch B of anti-C3. Values are metric means of four cultures $\pm$ s.e.m.

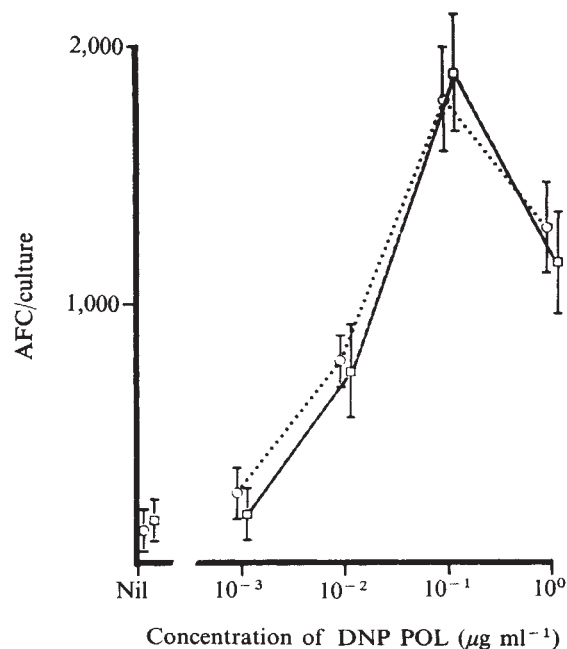


Fig. 3 Effect of anti-C3 on the response of spleen cells to DNP-POL *in vitro*. ○, Response to DNP-POL in presence of anti-C3; □, response to DNP-POL in presence of control IgG. Results taken from one out of three such experiments, using batch B of anti-C3. Values are metric means of four cultures $\pm$ s.e.m.

or both. For example, fixed C3 interacting with cell surface C3 receptors might assist in T-dependent presentation of antigen to B cells^{7,8}. The contrasting suggestion¹⁶ that interaction of C3 with B cell C3 receptors is an obligatory signal for triggering of all B cells by either mitogens or antigens is not supported by this or other published work^{7,22}. The recent observation of a difference between the B cell precursors responsive to DNP-POL and TNP-KLH, particularly in primed mice²³, might provide an alternative explanation of the present data if the difference could be correlated with heterogeneity of lymphocyte C3 receptors. A combination of cell separation and tissue culture techniques may help to clarify the role of C3 in antibody production.

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MARC FELDMANN

ICRF Tumour Immunology Unit,
Department of Zoology,
University College London,
Gower Street, London, WC1

M. B. PEPYS

Department of Pathology,
University of Cambridge

Received December 18, 1973.

* Present Address: Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London.

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Lymphocytes from human newborns abrogate mitosis of their mother's lymphocytes

THE conceptus is not automatically rejected by its mother as an allograft even though it differs genetically and therefore antigenically¹⁻³, perhaps in part because foetal tissues are segregated from immunologically competent maternal cells. For example, the surface of trophoblastic cells is partially covered by highly sulphated acid mucoprotein and the trophoblasts themselves have few if any transplantation antigens³⁻⁸. Also, blocking factors may prevent the mother from immunologically damaging her foetus(es). For example, pregnant mice have a serum factor which blocks their lymphocytes from inhibiting the growth of antigenically foreign mouse embryonic cells⁹; similar results have been observed with human materials^{10,11}. We now report a further possible mechanism by which the newborn actively suppresses mitosis of maternal and other foreign lymphocytes.

Blood was obtained from the umbilical cords of male singleton newborns after delivery but before expulsion of the placenta, from their mothers' arm veins within 1 h of delivery and from nonpregnant adult females and adult males (ages 21-43, all healthy). Lymphocytes were prepared primarily from blood defibrinated by glass beads and purified by Ficoll-Hypaque gradient centrifugation¹², and on occasion passage through glass wool columns. Using Türk's reagent, lymphocyte

TABLE 2 Results of mixing lymphocytes from human adults with lymphocytes from human newborns

Case identification	Mixed lymphocytes male	female	PHA-P (50 µg ml ⁻¹)	% of metaphase cells having Y chromosome
A.M. + A.M.	Newborn	His mother	+	99
A.M. + A.M.	Newborn	His mother	0	98
B.M. + M.C.	Newborn	Alien mother	+	90
B.C. + M.M.	Newborn	Alien mother	+	93
B.S. + A.J.	Newborn	Alien mother	+	90
B.C. + R.D.	Newborn	Nonpregnant adult	0	92
V.S. + B.C.	Newborn	Nonpregnant adult	+	88
B.J. + C.H.	Adult	Nonpregnant adult	+	54
B.J. + M.W.	Adult	Nonpregnant adult	0	60
M.O. + C.H.	Adult	Nonpregnant adult	+	53
Expected result	1:1 Mixture		+ or 0	50

Equal numbers of lymphocytes (1 to 2 × 10⁶) from male newborns or male adults and lymphocytes from adult females were mixed in culture. After 3 d incubation, the cells were treated with colchicine, collected and stained with quinacrine hydrochloride (to mark the Y chromosome). At least fifty cells in metaphase were examined from each culture and the percent having Y chromosome calculated. Under the culture conditions used, lymphocytes from males or females survived equally well.

purity was 95% or better as judged by light microscopy. Equal numbers of living lymphocytes from each mother and her newborn (1-2 × 10⁶ from each) were cultured in 5 ml of RPMI 1640 media supplemented with 20% foetal calf serum, L-glutamine and antibiotics. Cultures were set up in parallel with and without phytohaemagglutinin P (PHA-P) because most cells needed PHA to go into mitosis. Adult and newborn lymphocytes cultured separately with similar amounts of PHA served as controls. We used 50 µg of PHA per ml of medium, previously determined to be an optimal dose for both types of lymphocyte. After 60-72 h incubation at 37° C in 5% CO₂ and 95% air, cultures were treated with colchicine, collected and prepared as described before¹³. Slides were stained with 0.15% quinacrine hydrochloride, washed, mounted in modified McIlwaine's citric acid buffer¹⁴, examined by fluorescent microscopy and photographed, after which the karyotype was constructed. This technique marks the Y chromosome specifically. Under the culture conditions used, cells from mothers survived as well as cells from the newborns, as judged by trypan blue dye exclusion.

Table 1 shows that after 3 d culture of a mixture of lymphocytes most cells undergoing mitosis were from the newborn. With or without PHA, 98% or more of the total cultured peripheral lymphocytes in mitosis were the newborn's (as determined by the presence of the Y chromosome), whereas 2% or less were from the mother.

In further experiments the chromosome labelling technique was used to study the mixture of lymphocytes from newborns and adult lymphocytes other than those of their mother. In all cases, lymphocytes from male newborns inhibited mitosis of lymphocytes from unrelated mothers and nonpregnant adult females (Table 2). In contrast, lymphocytes of adult males failed to inhibit mitosis of lymphocytes from adult females. Viability of cultured lymphocytes from adults and newborns was again similar in each experiment. We were unable to assess the mixing of lymphocytes from newborn females with their mothers' lymphocytes because of similarities in chromosome markers.

These results clearly show that lymphocytes from human male newborns effectively inhibit division of their mothers'

TABLE 1 Results of mixing lymphocytes from human newborns with lymphocytes from their mothers

Case identification	PHA-P (50 µg ml ⁻¹)	% of metaphase cells having a Y chromosome
E.B.	+	100
J.E.	+	100
J.S.	+	100
V.V.	+	100
A.M.	+	99
A.M.	0	98
V.G.	+	100
V.G.	0	100
Expected result	+ or 0	50

Equal numbers of lymphocytes (1 to 2 × 10⁶) from the mother and her male newborn were mixed in culture. After 3 d incubation the cells were treated with colchicine, collected and stained with quinacrine hydrochloride (to mark the Y chromosome). Usually 100 cells in metaphase were examined from each culture for the presence or absence of the Y chromosome. Lymphocytes were refined by Ficoll-Hypaque gradient centrifugation and, on occasion, by passage through glass wool columns. Under the culture conditions used, lymphocytes from the mother survived as well as lymphocytes from the newborns.

lymphocytes *in vitro*; however, lymphocytes obtained from the mother and cultured separately were able to enter mitosis. Preliminary experiments suggest that a soluble factor released from the newborn's lymphocytes may be in part responsible for inhibition of mitosis. Further, this inhibition is a general phenomenon, in that lymphocytes from newborns also block division of lymphocytes from unrelated mothers and other adult females. In contrast, lymphocytes from adult males failed to abrogate mitosis of lymphocytes from adult females, suggesting that (1) the Y chromosome per se is not important in this inhibition and (2) the inhibitory effect is a property of the lymphocytes of newborns. No attempts were made to assess the suppression of maternal response by transplantation antigens. Others have shown that lymphocytes of both the newborn and its mother respond less to mitogen stimulation when mixed together in a unidirectional mixed culture relative to paternal or other third party cells^{15,16}. Experiments to be reported elsewhere show that lymphocytes from the mother are not killed when mixed only with lymphocytes from her newborn.

Our results suggest that any lymphocytes from the mother coming into contact with the foetus by transplacental passage are actively prevented from dividing. Such a process could protect foetal tissue from aggressor lymphocytes, prevent foetal rejection and explain the experimental difficulty in causing a graft *versus* host reaction in the foetus by cell transfer¹. While lymphocytes from human male newborns can inhibit mitosis of lymphocytes from their mothers, one cannot conclude from *in vitro* data that this is the sole reason why antigenically different embryos are not injured *in vivo*.

Finally, our results offer an explanation for earlier observations that immune responses were difficult to elicit in newborn rabbits after adoptive transfer of sensitised homologous adult lymphocytes, whereas such adult lymphocytes mounted sizable antibody responses after transfer to X-irradiated homologous adult rabbits^{17,18}. Perhaps lymphocytes from the newborn rabbits prevented potentially capable transferred cells from forming antibody. We are investigating whether lymphocytes from newborns can turn off a humoral response by preventing the division of primed immunocompetent cells and whether such lymphocytes are T suppressor cells.

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LARS B. OLDING

MICHAEL B. A. OLDSTONE

Department of Experimental Pathology,
Scripps Clinic and Research Foundation,
La Jolla, California 92037

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Effects of barbiturate withdrawal on audiogenic seizure susceptibility in BALB/c mice

BARBITURATES are commonly used to prevent epileptic seizures¹, but it has also been observed that susceptibility to 'spontaneous' seizure in both normal humans and animals is increased during abrupt withdrawal from chronically administered barbiturates and other central nervous system depressants². Here we show that barbiturate withdrawal is highly effective in producing audiogenic seizure susceptibility in a strain of mice which are normally resistant to seizure.

In addition, we present evidence that the barbiturate withdrawal effect is not an age-dependent phenomenon and further suggest that this drug-induced susceptibility to seizure shares features in common with the seizure susceptibility that can be induced in these same mice following a reduction of auditory input.

Audiogenic seizures, that is seizures driven by a loud sound, can be produced in normally seizure-resistant mice by 'priming' them with an intense acoustic stimulus a few days before testing for audiogenic seizures³⁻⁵. These primed animals show a severe loss in cochlear sensitivity indicative of cochlear damage. Saunders *et al.*⁶ proposed an auditory deprivation hypothesis to account for the apparent paradox that deafened mice over-react to a loud sound. Essentially, this hypothesis suggested that priming caused damage to the cochlea which in turn effectively reduced auditory input to higher auditory structures. As a result of this disuse, the unused structures became supersensitive so that on re-exposure to a loud sound, the supersensitive structures were excited and a seizure precipitated. Gates, Chen and Bock⁷ and Chen, Gates and Bock⁸ later confirmed that auditory deprivation was the important condition for the development of supersensitivity.

Careful destruction of the tympanic membrane produces a reduction of input to the cochlea (approximately 40 dB) and would not seem to produce cochlear damage. There is, however, the chance that secondary infection could be introduced into the middle ear and then to the cochlea, but this did not seem to be the case with our animals. McGinn *et al.*⁹ have subsequently confirmed our finding that a reduction of auditory input, by means of ear plugs, is also effective in producing audiogenic seizure-prone mice. Willott and Henry¹⁰ report that preliminary histology from primed mice localises damage in the outer hair cells of the cochlea. Although we have no histological evidence of hair cell damage from our BALB/c mice following priming, there is no reason to believe that priming does not produce the same effect as in C57BL/6J mice. The 'poor' cochlear microphonic obtained from BALB/c mice after priming is indicative of hair cell damage.

Disuse in the nervous system, regardless of how it is produced, seems to be the condition responsible for an increase in nervous system excitability¹¹. In view of the evidence

Table 1 Effects of barbiturate withdrawal on seizure susceptibility in non-susceptible BALB/c mice as a function of age

Group	Age	No.	Incidence of seizure pattern*			
			Wild running	Clonic	Tonic	Death
Barbiturate	36 $\pm$ 3	10	10	10	9	0
Saline	36 $\pm$ 3	6	1	0	0	0
Barbiturate	270 - 275	8	8	8	5	0
Saline	270 - 275	11	1	1	1	0

* Fisher exact probability tests were used to compare the barbiturate and saline control mice for each stage of the seizure pattern. Exact probability values are given in the text.

which suggests that barbiturate anaesthetics produce a general reduction in excitability in at least some parts of the auditory pathway¹² and as a result of the suggestion put forward by Jaffe and Sharpless¹³ (and others) that pharmacological disuse supersensitivity resembles other types of disuse supersensitivities, we decided to examine the effect of barbiturate withdrawal on susceptibility of BALB/c mice to audiogenic seizure.

Twenty-one mice from eight litters were divided into two groups (split litters) at 36 $\pm$ 3 d of age. This age group was the closest we could get to the immature mouse without introducing other difficulties. Each of the 14 experimental mice was given an intragastric dose of 1.8 mg (approximately 60 mg kg⁻¹) of sodium phenobarbitone dissolved in 0.3 ml of water twice daily at 12 h intervals for 5 d. The other group of seven mice was also given an intragastric dose of isotonic saline of the same volume on the same regimen. The barbiturate was given orally in both age groups. Four experimental and one control animal died during the treatment period. (We were concerned that interference with growth, through food deprivation, might introduce a confounding factor into the experiment but by lowering the dosage and increasing the time over which the drug is administered another problem is created—achieving the effect within the desired age group.)

Thirty hours after the last dose of phenobarbitone had been given to the experimental group, all mice from both groups were tested for susceptibility to audiogenic seizure by exposing each mouse to the sound of an intense electric bell (131 dB relatively to 0.0002 dyne cm⁻²) inside a circular, sound-insulated chamber for 60 s or until seizure occurred. The types of seizure reactions were noted in those mice which convulsed and the results are shown in Table 1.

All the animals that had been withdrawn from phenobarbitone had florid seizures, but only one of the control animals showed the first stage of the audiogenic seizure sequence; that is, wild running without subsequent seizure. Fisher exact probability tests on incidence of each level of seizure behaviour reveal significant differences between the drug and saline control groups (wild running, $P = 0.0014$; clonic seizure, $P = 0.00012$; tonic seizure, $P = 0.0009$). Barbiturate withdrawal is clearly effective in inducing seizure susceptibility in these normally seizure-resistant mice.

Production of audiogenic seizure susceptibility in resistant mice by previous auditory priming is often regarded as being a developmental phenomenon; that is, the mice can only be made susceptible to seizure if priming occurs during a sensitive period when the mice are still undergoing neural maturation. Gates and Chen¹⁴, however, have recently shown that seizure susceptibility can be achieved in adult mice if the intensity of the priming stimulus is greater than that used to prime younger animals. In view of this finding we thought it useful to examine the effects of barbiturate withdrawal on seizure susceptibility in mature mice.

Twenty-three mice from five litters were divided into two groups (split litters) of 12 and 11 respectively at 270–275 d

of age. Each of the 12 experimental mice was given a 60 mg kg⁻¹ intragastric dose of sodium phenobarbitone, dissolved in 0.3 ml of water, twice a day for 4.5 d and the 11 control animals were given an intragastric dose of 0.3 ml of isotonic saline on the same regimen. Thirty-four hours after the last dose of barbiturate had been administered, both groups were tested for susceptibility to audiogenic seizure using the method described previously.

The results of testing are shown in Table 1. Four of the animals in the test group died during barbiturate habituation. All of the remaining animals in this group exhibited audiogenic seizures during withdrawal and only one of the 12 control animals showed seizure behaviour. Fisher exact probability tests show that there are significant differences between the groups in incidences of wild running ($P = 0.0001$), clonic seizure ($P = 0.0001$) and tonic seizure ($P = 0.041$). Barbiturate withdrawal is therefore also an effective method for inducing seizure susceptibility in mature mice.

BALB/c mice are genetically resistant to audiogenic seizure. As we have already pointed out, however, susceptibility to seizure may be induced in these mice by exposure to either a loud sound or by depriving them of auditory input several days before the test. Fuller and Wimer¹⁵ and Freund and Walker¹⁶ have also shown that ethanol withdrawal is effective in inducing seizure susceptibility. Although all these methods of producing seizure susceptibility seem to be unrelated, they all share the common property of producing a depression of normal central nervous system activity. Priming produces cochlear damage which reduces input to higher auditory structures while auditory deprivation, by means of tympanic membrane destruction, has a similar effect. Phenobarbital and ethanol are both central nervous system depressants producing a reduction or depression of normal nervous activity. The major effect of reducing activity in the nervous system is that unused structures become supersensitive or over-reactive to sensory stimulation^{2,17}. On exposure to a loud sound, the supersensitive structures are driven by auditory input, either directly or indirectly, and a seizure is precipitated. The audiogenic seizure is merely the behavioural manifestation of this underlying supersensitive state.

The significance of our experiment is that we have been able to induce seizure susceptibility by pharmacological means in a strain of mice which can be made susceptible to audiogenic seizure by means of auditory disuse. The fact that auditory stimulation is effective in driving a seizure during barbiturate withdrawal indicates that such a model might be useful in determining whether or not the different methods of disuse share a common or similar mechanism of supersensitivity. Our model is easily prepared and tested and lends itself to a wide variety of biochemical manipulations. These results also prompt speculation about 'spontaneous' seizures which occur in other animals during barbiturate withdrawal; such seizures may be induced by sensory stimulation.

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G. R. GATES
C.-S. CHEN

Department of Psychology,
Monash University,
Clayton, Victoria 3168,
Australia

Received December 4, 1973.

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Correlation between euploid structural chromosome rearrangements and mental subnormality in humans

For some time cytogeneticists have been concerned with the question of whether structural chromosome rearrangements in humans, while apparently balanced, can have deleterious effects, either physical or mental. I wish to report data which suggest that the latter at least is possible.

Between 1959 and 1972 the chromosomes of blood leukocytes of 33,533 people were examined in the Medical Research Council Clinical and Population Cytogenetics Unit in Edinburgh, and ninety-four euploid structural rearrangements of the autosomes were found, apparently of independent origin. These consisted of thirty-eight Robertsonian translocations, forty-seven reciprocal translocations and nine pericentric inversions. The chromosomes of both parents of forty-six of the subjects were studied and thirty-four of the rearrangements were found to be familial, sixteen being paternal and eighteen maternal in origin, while twelve seemed to have arisen *de novo* and are referred to here as mutants (Table 1). Blood groups and red cell and serum enzymes were studied in all the mutants and their parents and there was no evidence that the legal parents were not the biological parents.

When these data were being compiled the proportion of

TABLE 2 Euploid structural rearrangements by method of ascertainment

Method of ascertainment	No. of individuals	Euploid rearrangements		
		No.	Both parents examined	No. of mutants
Mental subnormality (excluding Down's Syndrome)	2,426	12	7	5
Newborn survey	12,295	20	17	5
Other	18,812	62	22	2
Total	33,533	94	46	12

individuals who were mutants for a euploid chromosome rearrangement and who were also severely mentally retarded seemed to exceed chance expectation. To test this the data have been examined according to the method of ascertainment (see Tables 2 and 3 for details). At the time the original 33,533 blood samples were obtained the reason for the cytogenetic observation, that is, the method of ascertainment, was recorded for each patient. On the basis of this record the patients were divided into three groups: (a) those where the ascertainment was 'mental subnormality excluding Down's syndrome', (b) those where the ascertainment was 'consecutive or random liveborn babies', and (c) the remainder (Table 2). As the proportion of mutants in categories (b) and (c) was not significantly different ($P < 0.05$ —Fisher's exact test) the groups were combined and the proportion of mutants in the mental subnormality group was compared with the proportion among the remainder. The proportion of mutants among the mentally subnormal was significantly greater than the proportion among the other individuals ($P < 0.01$ —Fisher's exact test).

While 100% of the members of subnormal group were retarded, most being institutionalised and severely subnormal, several individuals in the other two groups must also have been mentally subnormal although only a very small proportion was likely to be severely retarded. In an attempt to see whether mutants in the other two groups were associated with mental subnormality, the method of discovery and clinical condition of the mutants in these classes were reviewed (Table 3).

Five of the seven mutants whose discovery was independent of any investigation of mental retardation were found during a survey of consecutive liveborn hospital births. One of the remaining mutants, who had a chromosome rearrangement t(3:20), (Table 3) was found during a survey of individuals occupationally exposed to ionising radiations, while the other, who had a rearrangement t(14q15q), was discovered during the investigation of a family in which a t(6p14p; 6q14q) rearrangement was segregating and it was shown that the t(14q15q) in the son must have been maternal in origin. It seems probable, especially as a break at or near the centromere of a chromosome 14 is involved in both translocations, that one rearrangement influenced the occurrence of the other¹.

All the newborn mutants appeared normal at birth, with the exception of laxity of the right hip in one baby (No. 173/71). This baby required hospitalisation at the age of 1 month because of myoclonic fits; however these were not observed in hospital and her EEG was normal. She is now well and at the age of 2 yr development seems to be normal. One child, 177/69, developed myoclonic epilepsy at the age of 3 months with a typical hypsarrhythmia pattern in his EEG. The fits were extremely difficult to control with medication and there was progressive mental deterioration until sudden death at the age of 3.5 yr. The remaining three mutant babies appear developmentally normal and the two mutant adults are physically and intellectually unremarkable.

TABLE 1 Euploid structural rearrangement of the autosomes

Type of rearrangement	Total No. observed	Total where both parents examined	Mutant	Inherited	
				Father	Mother
Robertsonian translocation	38	19	3	10	6
Reciprocal translocation	47	21	9	4	8
Inversion	9	6	0	2	4
Total	94	46	12	16	18

TABLE 3 Mutants ascertained independently of mental subnormality

Patient No.	Karyotype	Method of ascertainment	Phenotype
173/71	45, XX, t(14q21q)	Survey of consecutive liveborn babies	Laxity of right hip at birth. Physically and developmentally normal at 2 yr
26/69	46, XY, t(Cp-; Cq+)	Survey of consecutive liveborn babies	Normal at birth and reported by medically qualified parents to be normal in all respects at the age of 1 yr
177/69	46, XY, t(5; 11) (p11; p15)	Survey of consecutive liveborn babies	Normal at birth. Myoclonic epilepsy at age 3 months, progressive mental deterioration till death at age 3.5 yr
190/72	46, XX, t(2; 10) (q11; q22)	Survey of consecutive liveborn babies	Physically and developmentally normal at birth and at age 1 yr
231/72	46, XX, t(1; 15) (q2; q15 or 21 or 22)	Survey of consecutive liveborn babies	Physically and developmentally normal at birth and at age 6 months
228/72	45, XY, t(14q15q)	Son of chromosomally abnormal parent	Physically and mentally normal 27-yr-old unmarried male
14/71	45, XY, t(3; 20) (q29; p11)	Survey of males occupationally exposed to ionising radiation	Physically and mentally normal 47-yr-old male, with two children, both with a normal karyotype

TABLE 4 Published surveys of mentally subnormal individuals

Reference	No. of individuals examined (excluding Down's syndrome)	No. of euploid rearrangements	Both parents examined	No. of mutants
2	50	1	1	0
3	50	2	1	0
4	264	2	1	0
5	83	2	2	2
6	44	0	—	—
7	85	0	—	—
8	537	1	0	—
9	1,000	4	0	—
10	54	0	0	—
Total	2,167	12	5	2

Very few chromosome surveys of mentally subnormal individuals who do not have Down's syndrome have been published and, in almost every case where data are available, the individuals examined represent a highly selected subgroup of the mentally subnormal. The results of nine published surveys are given in Table 4 and while 12 of the 2,167 people examined were reported to have a euploid structural rearrangement, in only five were both parents examined. Two of these were found to be mutants and while these data are small, they agree with the observation that there is an excess of mutant euploid structural rearrangement among the mentally subnormal.

A deleterious effect of apparently balanced translocations in the heterozygous state has, as far as I am aware, no reported parallel in the animal kingdom. This may be because an experiment designed to detect such an effect would be virtually impossible with most laboratory animals. The phenomena may be widespread and many apparently balanced autosomal exchanges may have a deleterious effect in the heterozygous state on the cell and/or organism carrying them. The mechanism for such an effect could be either (a) that the rearrangement is in reality unbalanced and carries a small deletion below the level of cytological detectability; or (b) that chromosome breakage and exchange results in a gene mutation at one or both the break points; or (c) that certain rearrangements are associated with a position effect detrimental to their carriers.

Whatever the mechanism responsible, the data presented here suggest that the possession of a *de novo* euploid structural rearrangement of the autosomes is, in some cases, associated with and presumably causal to, severe mental retardation. However the follow-up of a large series of newborn babies who are mutants for a euploid structural rearrangement will be necessary before a precise prognosis for mental retardation can be made. More intensive study of mutant individuals might well enable us to determine not only the proportion of euploid mutations associated with

a phenotypic effect but also the mechanism by which the effect is produced.

PATRICIA A. JACOBS*

MRC Clinical and Population Cytogenetics Unit,
Western General Hospital,
Edinburgh

Received February 8, 1974.

* Present address: Department of Anatomy and Reproductive Biology, University of Hawaii School of Medicine, Honolulu, Hawaii 96822.

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Negative outcome of a blind assessment of the association between spray adhesive exposure and human chromosome breakage

THE recent claim of an association of exposure to spray adhesives with a rate of chromosome breakage five to six times that in control subjects, and the report of birth defects in two children of exposed parents led the United States Federal Consumer Product Safety Commission, in August 1973, to ban abruptly the sale of these products. They were recalled from the market and a widely publicised nationwide warning urged all people exposed, particularly pregnant women, to seek chromosome studies. This resulted in widespread popular concern. Cytogeneticists throughout the country received many requests for advice from exposed women concerning termination of pregnancy and future genetic risks. Evaluation of these requests was particularly difficult because there had been no formal publication of the original data at the time of the warning. (And as of January 15, 1974 none has appeared.) The only information released to investigators who queried the Product Safety Commission directly was a copy of a letter from J. R. Seely

TABLE 1 Chromosome breakage in individuals exposed to spray adhesives and in matched controls

	Study A		Study B		Both studies together	
	Exposed	Controls	Exposed	Controls	Exposed	Controls
No. of subjects	11	11	4	4	*	*
No. of metaphases per subject	~36	~36	72	72	*	*
Total No. of metaphases studied	397	398	288	288	685	686
Frequency (and %) of cells with evidence for breakage	7 (1.8%)	3 (0.8%)	2 (0.7%)	4 (1.4%)	9 (1.3%)	7 (1.0%)
	$P = 0.34$ ($\chi^2 = 0.92$)		$P = 0.68$ (Fisher's exact test, two-tailed)		$P = 0.81$ ($\chi^2 = 0.06$)	

Phytohaemagglutinin stimulated peripheral blood was used; the total time in culture was 72 h because in all experiments collection⁵ at 48 h revealed growth too sparse to provide adequate numbers for counting. (The Oklahoma report also used cells cultured for 72 h.) Our procedure included treatment with hypotonic KCl, air drying of cell preparations dropped onto cold water-wet slides from a distance of 18 inches, and staining with carbol fuchsin. Total time in colcemid ($0.5 \mu\text{g ml}^{-1}$) was 2 h. Unstained areas in chromatids, no matter how large, were considered gaps not breaks, unless there was a clear discontinuity of the chromatid as well as nonalignment of the chromatid axis. Cells with gaps only were not scored as having evidence for chromosome breakage. Study A lasted for 2 weeks, and one batch of media was used. Exposed and control subjects could not always be set up on the same day. Only metaphases with at least forty-four centromeres were scored. In the exposed group there was one cell with a dicentric chromosome and six others, each with an acentric fragment. The dicentric and one fragment occurred in the same individual. Otherwise no more than one break occurred in any individual studied. In the controls there were two cells with isochromatid breaks and one with an acentric fragment. (There was also one dicentric in a control cell with only forty-one centromeres, and hence not scored.) In study B, blood from each exposed individual and matched control of a pair was drawn, cultured and studied at the same time. The specimens were coded at the time of sampling, and the code was not broken until after analysis. There was an interval of about 1 month between the sampling of first and last pairs. Individuals who were resampled from the original eleven included three of the four with heavy exposure (the fourth was unavailable) and one individual chosen randomly from the three with intermediate exposure. (The group resampled included the exposed individual in the first study with the dicentric and an acentric fragment. In none of the controls resampled had a break been found in any cell examined in study A.) In study B, two cells, each with an acentric fragment, were found in those exposed and one cell with a dicentric and three others with an acentric fragment were found in the controls.

* Seven pairs were studied once, (~36 metaphases from each subject), and four pairs twice (~108 metaphases from each subject). See text and footnote above.

at the University of Oklahoma who had made the original claim.

This evidence offered in support of the association we found suggestive, but hardly convincing, for the studies did not involve a blind assessment of chromosome breakage. The claimed relationship of birth defects to chromosome breaks and spray adhesive exposure was obscure moreover, for one of the two malformed children with alleged breaks had been conceived several months after the parents ceased use of the spray. In addition, because many of those exposed knew each other, even if there were an increased rate of chromosome breakage, this might have been caused by some other shared environmental factor.

We undertook a study of the problem in September 1973. Eleven individuals with histories of recent exposure to spray adhesives were matched with controls of the same age and sex. Blood specimens were obtained from each, coded, and set up in culture (Table 1). Approximately thirty-six metaphase plates were examined from each individual, providing a total of about 400 cells for analysis from each group. (The anticipated background rate of cells with chromosome breakage in adult controls from earlier studies was about 1.2%. Therefore, should equal rates at this level have been found in both groups, this observation would have excluded, with 95% confidence, a two-fold or greater increase over expected control rate.) When the code was broken, the results (study A, Table 1) revealed no significant difference between the two groups. In fact the observed frequency of breaks in the exposed subjects excluded with 95% confidence a rate five times or greater that observed in the controls, which had been the order of magnitude of the increase reported by the Oklahoma investigator. We then repeated the study on four of the original eleven subjects studied who were among those with the heaviest exposure (study B, Table 1). The controls were the same. Seventy-two metaphases from each individual were examined. Again there was no statistically significant difference between exposed and control subjects, and the results in the exposed excluded with 95% confidence a rate twice or more that observed in the controls.

No experiment can ever prove negative results, that is the absence of a difference between exposed and controls. The results can only put boundaries on the confidence limits

of the observed rates in a particular group of people with a particular history of exposure. Our observations, however, provide no grounds to reject the null hypothesis. That is, on the basis of our results, we believe there is no reason now to accept a purported association of significant magnitude between chromosome breakage and exposure to spray adhesives.

(While our manuscript was in preparation the Consumer Product Safety Commission announced the ban would be lifted¹. It has been reported that a blind reevaluation of the original slides failed to confirm the initial interpretation², and that other investigators, as well as ourselves, could not confirm the original report².)

This episode raises questions as to what evidence of chromosome breakage in humans should be grounds for action that may provoke unnecessary public alarm and adverse economic effects.

ERNEST B. HOOK
NORMA H. HATCHER

Human Ecology Section,
Birth Defects Institute,
New York State Department of Health,
Albany, New York 12208 and
Department of Pediatrics,
Albany Medical College of
Union University,
Albany, New York 12208

PAULINE S. BRINSON
ORYSIA J. STANECKY

Department of Pediatrics

LESLIE FISHER

Burns Care Institute,
New York State Department of Health

GERALD FECK
PETER GREENWALD

Cancer Control Bureau,
New York State Department of Health

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Linkage between immune response potential to DNA and X chromosome

INTEREST in the regulation of the immune response to nucleic acids stems largely from the spontaneous occurrence of anti-nucleic acid antibodies in the sera of patients with systemic lupus erythematosus¹⁻⁴. Genetic factors seem to play a role in this disease, which is associated with certain human transplantation antigens⁵. Spontaneous production of antibodies to different forms of nucleic acids has been observed in some mouse strains, and is suggested to be correlated with autoimmune diseases^{1-4,6-8}. It is of interest to find out whether the response potential of different mouse strains to nucleic acids, on active immunisation, is related to the spontaneous occurrence of antibodies and/or to autoimmune-like diseases.

We have previously reported that various mouse strains differ in their ability to respond to denatured DNA⁹. Thus, DBA/2 mice were found to be low responders to denatured DNA, whereas SJL/J mice were high responders to this immunogen. The SJL mice are of special interest, as they develop spontaneous reticulum cell neoplasms which resemble Hodgkin's disease in man¹⁰, as well as some immunological abnormalities including the production of anti-nuclear antibodies^{7,11}. It was found that the low responsiveness of DBA/2 mice to denatured DNA, is a result of their inability to form antibodies of the IgG class. SJL mice produced anti-denatured DNA antibodies of both 7S and 19S classes, whereas DBA/2 mice elicited only 19S antibodies⁹.

Here we demonstrate that the ability to respond to denatured DNA is genetically regulated by an X-linked gene carried on the X chromosome.

SJL mice, DBA/2 mice, their (SJL × DBA/2)F1 hybrids and the backcrosses of the SJL and DBA/2 females with F1 males, namely, SJL × F1 (SJL × DBA/2) and DBA/2 × F1 (SJL × DBA/2), were injected intraperitoneally with 150 µg denatured DNA in a complex with equal weight of methylated bovine serum albumin (MBSA).

The mice were immunised twice within a 3 week interval. The first immunisation was given in complete Freund's adjuvant, whereas in the second one the denatured DNA-MBSA complex was given in phosphate-buffered saline (0.01 M sodium phosphate buffer, pH 7.0, 0.15 M NaCl). The mice were bled 10 d after the second injection, and their sera were tested for anti-DNA titres by the antigen binding capacity assay, using ¹⁴C-DNA, as previously described⁹. In addition, mice of the backcross DBA/2 × F1 (SJL × DBA/2) were assayed for their H-2 antigenic specificities. The animals were typed by the haemagglutination method^{12,13}, using anti-H-2^{s/s} and anti-H-2^{d/d} alloantisera^{13,14}.

The immune response to DNA of the animals described above, after the second injection, is demonstrated in Fig. 1. As was shown earlier⁹, the SJL mice are high responders, whereas DBA/2 mice are low responders. The F1 hybrids are intermediate responders. The backcross animals, DBA/2 × F1 (SJL × DBA/2) and SJL × F1 (SJL × DBA/2), segregate in their response as expected from the response potential of the parental strains. Thus, the ability to respond to denatured DNA is genetically controlled.

Typing of the backcross mice DBA/2 × F1 (SJL × DBA/2) for their major histocompatibility (H-2) specificity indicated that the capacity to respond to denatured DNA is not linked to H-2, as the H-2^s antigenic specificity, donated by the SJL high responder strain, was detected in low and high responder animals. The results obtained with these backcross mice, however, demonstrate that the gene which regulates the response potential to DNA is on the X chromosome. All the males of the DBA/2 × F1 (SJL × DBA/2) backcross were low responders, whereas all the females responded well (Fig. 1). These results could be expected if the response to DNA is controlled by a dominant X-linked gene. All the males of this backcross possess the *X_{low}* allele donated by the

DBA/2 low responder females. On the other hand, all the females carry one *X_{high}* allele donated by the parental males F1 (SJL × DBA/2). These F1 parental males carry the *X_{high}* allele, as they are offspring of a cross between SJL high responder females (*X_{high} X_{high}*) and DBA/2 low responder males (*X_{low} Y*). Mice of the backcross SJL × F1 (SJL × DBA/2) were good antibody producers (Fig. 1), as expected, as all of them carry at least one *X_{high}* allele. Thus, the females of this backcross are *X_{high} X_{high}* and the males are *X_{high} Y*.

Analysis of the antibody classes produced by mice of the backcross DBA/2 × F1 (SJL × DBA/2) indicated that a poor responsiveness to DNA is associated with inability to elicit antibodies of the IgG class, as was previously shown for the DBA/2 low responder parental strain⁹. Thus, the low responder males of this backcross produced only 19S antibodies, whereas in the sera of the high responder females, antibodies of both 19S and 7S were present.

The immune response to another nucleic acid, namely, double-stranded polyinosinic-polycytidylic acid, (poly(I)·poly(C)), was also found to be controlled by a gene on the X chromosome¹⁵. It has been suggested that poly(I)·poly(C) is a thymus-independent antigen, as neonatally thymectomised mice produced antibodies to this antigen¹⁶. The immune response to type III pneumococcal polysaccharide, which is a thymus-independent antigen, is also linked with the X chromosome¹⁷. Is the response potential to denatured DNA thymus independent, and if so, is an association between the

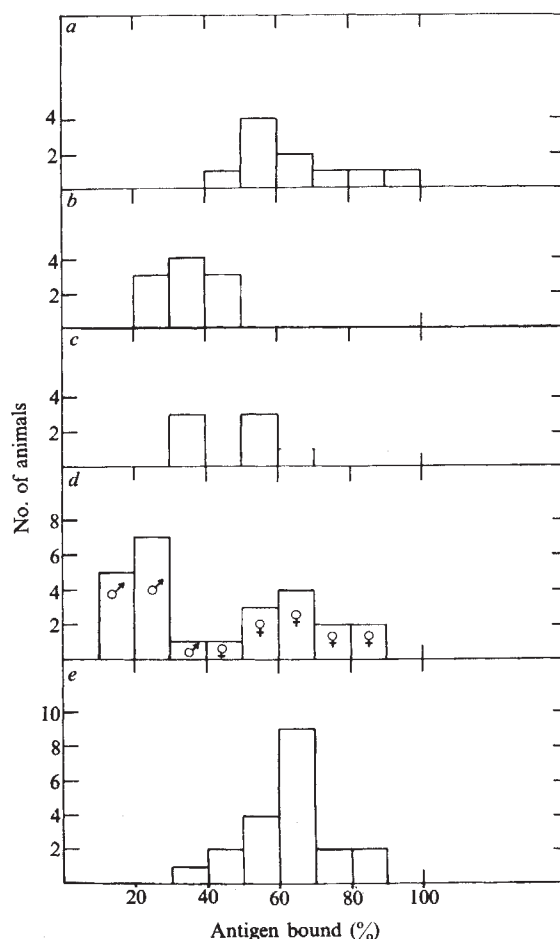


FIG. 1 The antibody response of mice immunised with denatured DNA-MBSA and assayed with ¹⁴C-DNA. The horizontal axis plots the % antigen bound in the antigen-binding assay, while the vertical axis plots the number of animals falling into a given percentile for antigen bound values. 10 µl of each antiserum were assayed. a, SJL; b, DBA/2; c, (SJL × DBA/2) F1; d, DBA/2 × F1 (SJL × DBA/2); e, SJL × F1 (SJL × DBA/2). The sex of the animals is shown in d.

gene(s) controlling the response to thymus-independent immunogens and the X chromosome a general phenomenon?

An efficient immune response to denatured DNA could be obtained only when the immunogen was complexed with MBSA. Strain-dependent differences in the response potential to some synthetic polypeptides^{18,19} have been shown to be obliterated when the immunogens were injected as complexes with MBSA. In the case of denatured DNA, strain differences in the ability to produce antibodies were observed following immunisation with complexes of denatured DNA-MBSA.

Here we have demonstrated a linkage between the ability of mice to respond to denatured DNA upon active immunisation, and the X chromosome. Such a linkage may contribute to the elucidation of the phenomenon of autoimmune diseases, especially if a similar association should also be found between spontaneous antibodies to DNA and the sex chromosome. This experimental approach may be of relevance for the understanding of human immune disorders, as the incidence of some autoimmune diseases in man seems to be associated with sex^{20,21}.

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EDNA MOZES
SARA FUCHS

Department of Chemical Immunology,
The Weizmann Institute of Science,
Rehovot, Israel

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Lymphocyte separation from blood

MUCH attention has been focussed on the role of lymphocytes in the immune response of the tumour-bearing host. To study the cytotoxic effects of lymphocytes from rabbits bearing VX2 tumours towards the tumour cells *in vitro*, a suitable method for obtaining lymphocytes from peripheral blood was sought. With the recognition that both T and B lymphocyte populations are heterogeneous, and have different properties and functions which modulate each other's activities¹, the question of cell yield assumes importance in lymphocyte preparations. The method of choice should also be simple and rapid.

Several methods have been described in the literature for preparing comparatively pure lymphocyte suspensions from peripheral blood. For separation of lymphocytes from granulocytes, columns of cotton wool or glass beads have been widely used²⁻⁴. This method is rather lengthy and is difficult to carry out in sterile conditions. The major disadvantage is that erythrocytes are not efficiently retained by the columns, and the effluents always contain a similar or higher number of erythrocytes as lymphocytes. The yield of lymphocytes recovered by this method is usually small (about 50%). Purification of lymphocytes from blood by either Triosil (Iso-paque)-Ficoll or carbonyl iron powder seemed to be simple and less time consuming, and these two methods were examined for preparing lymphocytes from rabbit blood.

Böyum⁵ devised the Triosil-Ficoll method for separating lymphocytes from human blood. His lymphocyte preparation usually contained about 80% lymphocytes, 18% monocytes and 6% erythrocytes with a lymphocyte yield of greater than 95%. Harris and Ukaejiofo⁶ using the same method, obtained on average 70% of the available lymphocytes. Using this Triosil-Ficoll method, we obtained lymphocyte yield from human blood of between 30% and 40% and from rabbit blood a yield of between 6% and 50%. Attempts to obtain higher yields were not successful. In addition, the purity of the lymphocyte preparations was not satisfactory.

Several groups of workers have used the carbonyl iron method for preparing lymphocyte suspensions⁷⁻⁹. These workers do not state the actual yield of lymphocytes recovered per ml of blood, although from the results of Coulson and Chalmers⁷ it can be calculated that their yield for human blood was 25-50%. A lymphocyte yield from rabbit blood of greater than 70% (average 74%) has been consistently obtained by us with this technique. The yield of lymphocytes obtained from human blood, however, was always about 40%. The carbonyl iron method is simple and the entire purification procedure requires less than 2 h.

Venous blood (10 ml) was placed in a siliconised universal bottle containing 200 units of phenol-free heparin (Heparin BP (Mucous), Boots Pure Drug Co. Ltd., Nottingham, England) in 1 ml of saline. To this, 3.3 ml of sterile 1% methyl cellulose (viscosity 15 centipoise, Standard Grade, Dow Chemical Co.) in saline containing 120 mg of sterile carbonyl iron powder 3 μ m size (SF, GAF, Great Britain Ltd., Manchester) was added. The universal bottle containing the blood-methyl cellulose-iron mixture was incubated at 37°C for 30 min and was gently shaken at 5 min intervals. At the end of 30 min incubation, the contents of the bottle were transferred to another siliconised universal bottle which was left on a magnet at 37°C for 30 min. Red cells, granulocytes and monocytes settle with the iron particles. The supernatant, containing mainly lymphocytes and some iron particles, was pipetted off into a fresh universal bottle using a sterile Nylon Cannula (Portex Ltd., Kent, England) attached to a 1 ml syringe. The red cell pellet was spun at low speed (1000 r.p.m.) for 30 s using an MSE bench centrifuge. The supernatant was carefully pipetted off as above and added to the previous supernatant. The lymphocytes from the combined supernatants were centrifuged (2000 r.p.m. for 5 min on MSE

bench centrifuge), and washed three times with warm Waymouth medium (Medium MB 752/1, Difco Laboratories). During this washing procedure, the lymphocytes are freed from residual iron particles by resuspending the cells in the medium without disturbing the iron deposit, and decanting to a fresh siliconised universal glass bottle. Finally, the lymphocytes were suspended in Waymouth medium containing 5% bovine serum albumin previously warmed to room temperature. The differential count was performed using white blood cell (WBC) fluid (1% acetic acid in saline with two drops of methyl blue).

The lymphocyte preparation from rabbit blood contains over 95% lymphocytes compared with 50–60% lymphocytes in the blood before separation. One or two erythrocytes are usually present for every five lymphocytes, as observed by Giemsa staining. The preparation of human blood obtained by this method contains 50–60% lymphocytes compared to 30–40% lymphocytes in the original blood. Usually one or two erythrocytes are present for every lymphocyte in the suspension.

Lymphocytes prepared as above from rabbits bearing VX2 tumours are currently being investigated for cytotoxic activity towards the tumour cells previously labelled by ^{125}I -iododeoxyuridine by *in vitro* or *in vivo* methods.

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S. A. SHAH
J. A. DICKSON

Cancer Research Unit,
Department of Clinical Biochemistry,
Royal Victoria Infirmary,
Newcastle-upon-Tyne

Received January 17, 1974.

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T lymphocyte requirement for MSV tumour prevention or regression

ADULT mice injected with murine sarcoma virus Moloney isolate (M-MSV) develop sarcomas which have a high incidence of spontaneous regression. As tumour cells are strongly antigenic and release virus continuously, it is generally agreed that tumour regression is mediated by a host immune reaction¹. In fact, M-MSV induces a progressively growing tumour in newborn or adult mice immunologically depressed by X-irradiation, anti-lymphocyte serum or neonatal thymectomy^{2–4}. It is still not clear, however, if humoral or cellular immunity is mainly responsible for M-MSV tumour

regression^{5–8}. Both bone marrow-derived (B) and thymus-derived (T) lymphocytes have been shown to possess a killer effect *in vitro* against target cells bearing Moloney virus specified cell surface antigens^{9,10}. Here we report preliminary data which indicate that T lymphocytes are necessary for spontaneous M-MSV tumour regression.

CBA mice of both sexes were thymectomised at 2 months of age. Two weeks later they were both irradiated with 850 rad and injected intravenously with 5×10^6 syngeneic bone marrow cells. These mice were then considered 'deprived' for T cell functions. A cell-free tumour extract of M-MSV, maintained by serial *in vivo* passage in 1–2-week-old CBA or BALB/c mice, was prepared as previously reported⁸. Three to four weeks after irradiation, deprived mice were injected intramuscularly in the left thigh with 0.05 ml of this tumour extract diluted to 10^{-2} g equivalent. Control CBA mice were similarly treated. All mice were inspected daily for tumour development and growth.

As shown in Table 1, 27 of 30 deprived mice (90%) developed tumours with a mean latent period of 17 d; moreover, 24 of these 27 mice died with progressive tumours 2–3 months following M-MSV injection. No tumours were observed in M-MSV-injected control mice. These results indicate that the deficiency of the T cell population is responsible for the induction and progression of tumours in the deprived mice.

To better evaluate whether restoration of T cell function could reverse the oncogenic response, deprived mice were grafted under the kidney capsule with neonatal thymus lobes, either from CBA or CBA/H-T6T6 donors, at different time intervals before or after M-MSV injection. These mice were considered 'reconstituted' for T cell function. Reconstitution was also evaluated in mice grafted with CBA/H-T6T6 thymuses, by means of karyotypic analyses of peripheral blood lymphocytes after PHA stimulation¹¹. As seen in Table 2, mice receiving the thymus graft 30 d before M-MSV injection did not develop tumours while mice which were grafted 1, 5 or 25 d after M-MSV injection presented a tumour incidence of 87.5, 69 and 100%, respectively, with a latency of 15–19 d. Furthermore, the mice reconstituted 1 or 5 d following M-MSV injection presented 100% regression while the mice grafted with a thymus 25 d after M-MSV injection presented tumours which progressed and killed the host.

It has been reported that following thymus grafting in deprived mice, a gradual restoration of T cell functions occurs and is accomplished in about 30 d (ref. 12). Consequently, our results may easily be explained in terms of complete or partial restoration of T-cell activity at the time of M-MSV injection. Thus, the mice grafted before M-MSV injection were as immunocompetent as the controls and did not develop tumours. Mice grafted 1 or 5 d after M-MSV injection present tumours but all of them regress, most likely because of a gradual increase in the T cell population during tumour growth. In this regard, the time needed for regression was greater (31 d) for mice grafted on the fifth day than that needed for mice grafted on the first day following M-MSV injection (13 d). Finally, thymus grafting 25 d after M-MSV does not affect the tumour because the neoplasm has probably reached a very advanced stage.

TABLE 1 Tumour induction by M-MSV in normal and deprived CBA mice

	Total No. mice	No. mice with tumour (%) [*]	No. mice with regressed tumour (%) [†]	No. mice dead with tumour (%) [‡]
Normal	30	0 (0)	0 (0)	0 (0)
Deprived	30	27 (90)	3 (11)	24 (89)

^{*} % of mice with tumour evaluated from total number of mice.

[†] % of mice with regressed tumour calculated from number of mice developing tumour.

[‡] % of mice dead with tumour calculated from number of mice developing tumour.

TABLE 2 Tumour induction in CBA mice thymus-grafted at different intervals before or after M-MSV injection

Time of thymus graft (d)	Total No. mice	No. mice with tumour (%) [*]	No. mice with regressed tumour (%) [†]	No. mice dead with tumour (%) [‡]
-30	14	0 (0)	0 (0)	0 (0)
+1	8	7 (87.5)	7 (100)	0 (0)
+5	13	9 (69.2)	9 (100)	0 (0)
+25	6	6 (100)	0 (0)	6 (100)

^{*} % of mice with tumour evaluated from total number of mice.

[†] % of mice with regressed tumour calculated from number of mice developing tumour.

[‡] % of mice dead with tumour calculated from number of mice developing tumour.

TABLE 3 M-MSV neutralisation titres^{*} of sera from normal, deprived and reconstituted mice at different days following M-MSV injection

	Days from M-MSV Injection		
	15	20	60
Normal	8	16	32
Deprived	4	8	16
Reconstituted	—	16	32

Pools of sera inactivated at 56°C for 30 min, were incubated at various dilutions for 60 min at room temperature with 30-40 focus forming units (f.f.u.) of M-MSV. The mixtures of sera and M-MSV were then assayed for focus formation on DEAE-dextran treated 3T3FL mouse cells plated the day before at concentration of 10⁵ in 60 mm plastic petri dishes. The cultures, in duplicate, were grown in Dulbecco's medium supplemented with 10% inactivated and filtered foetal calf serum and antibiotics. Foci were scored on the fifth day.

^{*} Reciprocal of serum dilution that resulted in a 50% reduction of focus formation is given.

As there is evidence indicating that humoral antibodies may interfere with M-MSV tumour induction and progression^{5,7,13}, it was considered worthwhile to study whether deprived and reconstituted mice could produce virus-neutralising antibodies. Mice, normal, deprived and reconstituted before M-MSV, were bled at various intervals following M-MSV injection. Their sera were assayed by the *in vitro* focus reduction method on 3T3 FL cells¹⁴. As shown in Table 3, both deprived and reconstituted mice produced neutralising antibody, although the former had a somewhat lower titre. This last finding could be due to the decrease of antibody in free form, which is usually observed in the serum of mice with progressing tumours⁹. Alternatively, since both 19S and 7S immunoglobulins are responsible for virus neutralisation¹⁵, it might be that the capability to synthesise 7S antibodies, which is a function of T and B cell cooperation¹⁶, is reduced or absent in deprived mice. Obviously, the fact that deprived mice produce virus-neutralising antibody does not exclude the possibility that their serum contains also antibody with an enhancing or blocking effect, although these antibodies are mainly of the 7S type¹⁷. The importance of T cells in restraining M-MSV oncogenicity clearly emerges in the experimental model studied. However, whether T cells operate directly by a cytotoxic effect on tumour cells or through cooperation with B cells resulting in more efficient antibody production, remains to be elucidated.

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D. COLLAVO
A. COLOMBATTI
L. CHIECO-BIANCHI

Laboratory of Experimental Oncology,
Institute of Pathological Anatomy,
University of Padova

A. J. S. DAVIES

Chester Beatty Research Institute,
London

Received January 14, 1974.

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Induced redistribution of membrane particles, anionic sites and con A receptors in *Entamoeba histolytica*

THE extent to which the distribution of antigens and other receptors at the cell surface is determined by structural components within the plasma membrane is largely unknown. In erythrocyte ghost membranes, freeze-fracture and freeze-etch studies have demonstrated that structural components, 'membrane intercalated particles', are the exclusive sites at the surface which bear A and B antigens^{1,2}, wheat agglutinin³, influenza virus³, con A receptors (Pinto da Silva and Nicolson, unpublished), and anionic sites⁴. It is clear, however, that conclusions derived from the study of this membrane system cannot be freely extrapolated to living cells where the relationship cell surface-membrane structure may be more complex. We report a study of this relationship on the plasma membrane of living *Entamoeba histolytica* cells in conditions which induce a redistribution of membrane intercalated particles, anionic sites or con A receptors.

Trophozoites of *E. histolytica*, a pathogenic strain cultured under axenic conditions⁵, were washed with phosphate-buffered saline (PBS). Aggregation of membrane particles was induced by gradual impregnation with 25% glycerol in PBS, incubation for 30 min at 34° C and 30 min more at

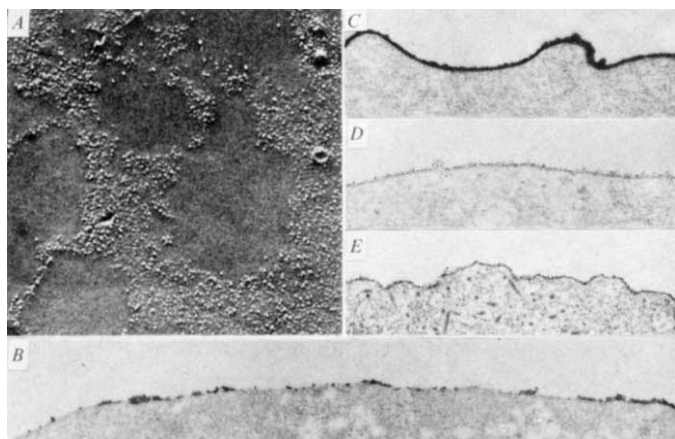


FIG. 1 Membrane intercalated particles, anionic sites and con A receptors in *E. histolytica* plasma membranes. A, Freeze fracture; B, colloidal iron at pH 1.8; C, con A-peroxidase; D, ferritin at pH 4.0; E, colloidal iron, prefixed cell. A, and D, 33,000; B, C and E $\times 16,500$.

24° C. Cell viability of glycerol impregnated cells as determined by exclusion of trypan blue remained over 75%, dead cells being easily distinguished in thin sections or in freeze-fracture replicas. For freeze fracture, cells were rapidly frozen in the liquid phase of partially solidified Freon 22, and fractured at -100°C in a Balzers 300 apparatus equipped with a turbomolecular pump. The ultrastructural distribution of acidic sites ionised at pH 1.8 was determined by labelling the cells with colloidal iron⁶. Negatively charged sites ionised at pH 4.0 were labelled with positively charged ferritin, obtained by dialysis at pH 4.0, that is, below its isoelectric point⁷. Con A receptors were detected by the con A-peroxidase reaction⁸.

To alter the distribution of structural components within the plane of the plasma membrane, living cells were incubated in glycerol, which results in aggregation of the membrane intercalated particles revealed by freeze fracture⁹. In our experimental conditions, we find that glycerol-induced aggregation of membrane particles in *E. histolytica* (Fig. 1A) is more prominent than that previously reported for lymphoid cells^{9,10}. Glutaraldehyde prefixation completely prevents glycerol-induced aggregation of membrane particles. In consequence, the aggregated pattern of membrane particle distribution which is observed on the fracture faces of plasma membranes is clearly distinguished from the non-aggregated pattern observed in cells fixed before glycerol impregnation. As can be seen in other cells systems, detailed examination of uncontaminated replicas of prefixed cells demonstrates, under favourable shadowing conditions, the existence of an extremely heterogeneous particle population where all transitions ranging from a slight rugosity—here termed ‘sub-particle’—to a definite membrane particle can be observed. This renders difficult the unequivocal identification of membrane particles. Aggregation of membrane particles induced by glycerol, however, provides a clear distinction between smooth and particulate regions of membrane fracture faces, because both particles and subparticles coaggregate into a clearly delimited network, which leaves in between regions denuded of any rugosity. The proportion of membrane area which is occupied by aggregates of particles and subparticles varies, even within a given cell, from 30 to 70%.

Ultrastructural observation of glycerol-pretreated cells demonstrates a patchy distribution of acidic sites labelled by colloidal iron at pH 1.8 (Fig. 1B). This contrasts sharply with the continuous and uniform distribution of both con A receptors (Fig. 1C) and acidic sites ionised at pH 4.0 (Fig. 1D). In control cells prefixed in glutaraldehyde, uniform

labelling over the entire cell surface is observed with all three reactions, as is illustrated in Fig. 1E for colloidal iron. Cells treated with ferritin, at pH 7.0, remain unlabelled.

In view of the report that con A induces aggregation of membrane particles in plasmocytoma cells¹¹, and the high agglutinability of pathogenic strains of *E. histolytica* relative to strains isolated from asymptomatic carriers¹², we studied the effect of con A on the distribution of membrane particles in pathogenic HK9 trophozoites. In our experimental conditions (5, 10, 20, or 100 $\mu\text{g ml}^{-1}$ con A, 30 min, 34° C) the lectin does not cause any apparent alteration in the distribution of membrane particles. In addition, treatment of cells with con A does not result in redistribution of membrane receptors detected with the peroxidase–benzidine reaction. When cells are fixed after con A–peroxidase treatment, however, the electron-dense precipitate is confined to a cell region containing the uropod, in a manner analogous to ‘cap’ formation in lymphocytes¹³, as will be reported in detail elsewhere. Freeze-fracture examination of these cells does not reveal a corresponding accumulation of membrane particles.

Our results demonstrate that glycerol treatment of *E. histolytica* cells results in marked aggregation of the membrane intercalated particles and in patchy distribution of acidic sites labelled by colloidal iron at pH 1.8, that glycerol induced aggregation of membrane particles is not accompanied by a redistribution of acidic sites ionised at pH 4.0 or of con A receptors and that con A receptors may redistribute independently of the membrane particles.

Our observations indicate that the cell surface–membrane structure relationship in *E. histolytica* cells is not straightforward. Although it remains possible that anionic sites labelled at pH 1.8 may correspond to the membrane intercalated particles, no such direct relationship exists between either acidic sites ionised at pH 4.0 or con A receptors and the membrane intercalated particles.

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PEDRO PINTO DA SILVA*

ADOLFO MARTINEZ-PALOMO

Centro de Investigación y de Estudios Avanzados del IPN,
Department of Cell Biology,
Apartado Postal 14-740,
México 14, DF México

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* On leave from The Salk Institute for Biological Studies, San Diego, California.

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Platelet secretion induced by divalent cation ionophores

SECRETION by platelets is stimulated by agents of diverse structure such as thrombin, collagen and adrenaline; the substances secreted include adenine nucleotides, 5-hydroxytryptamine and calcium¹. In many cells secretion seems to be mediated by the influx of Ca^{2+} (ref. 2), but this mechanism has not been demonstrated for all secretory systems and, in fact, no extracellular calcium is required for secretion by platelets. From kinetic studies of thrombin-stimulated secretion of ATP and calcium^{3,4} we proposed that stimulus-secretion coupling in platelets is mediated by intracellular calcium. We have now tested this hypothesis with the divalent cation ionophores X-537A (Lasalocid, Hoffman-La Roche) and A23187 (Lilly). These antibiotics increase the permeability of membranes to divalent cations and can perturb intracellular cation gradients⁵⁻¹⁰. The probable mechanism involves formation of lipophilic chelate compounds¹⁰. If secretion by platelets were mediated by the intracellular release of stored calcium (or some other divalent cation) the ionophores should disrupt the intracellular ion gradients and trigger secretion. This was the observed result.

Secretion was observed as the release of ATP, which was continuously measured by the firefly luminescence method⁴. The experiments were performed in the absence of extracellular Ca^{2+} , but addition of 1 mM Ca^{2+} or Ca^{2+} chelators did not affect the reactions. Both ionophores cause release of ATP with a time course that is nearly identical to that of thrombin-induced secretion (Fig. 1). The dependence on concentration of ionophore (Fig. 2) is consistent with other studies that show that A23187 is more effective as a calcium ionophore than is X537A (refs 5 and 7). Since the rate constant for release (Fig. 1) and the amount released (Fig. 2) are the same whether induced by thrombin or ionophores, the final steps in the multistep stimulus-secretion mechanism must be the same. In addition, no further ATP can be released by thrombin after treatment with ionophores and, conversely, ionophores do not release additional ATP from thrombin-treated platelets.

X537A and A23187 also cause release of calcium. We attempted to follow the kinetics of this release by the murexide dye binding technique³ but this method gave spurious results in this system. The apparent yield of calcium estimated

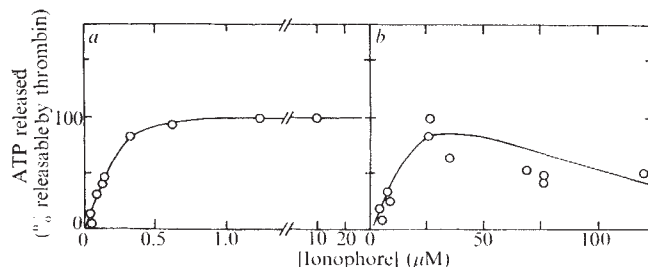


Fig. 2 Dependence of ATP secretion on concentration of ionophore. Yields are shown as percentage of ATP released by 4 U ml^{-1} of thrombin from the same platelet preparation. a, A23187; b, X537A.

from change in optical density of murexide was about twice the amount directly measured by atomic absorption. This error is probably due to binding of murexide to the membrane, a less polar environment. (We have measured large spectral shifts in murexide and calcium-murexide in non-polar solvents such as acetonitrile-water mixtures.) Atomic absorption measurements indicated that after 2 min exposure to ionophore nearly all the platelet calcium was recovered in the supernatant.

Interpretation of these results requires consideration of the known biological activity of these ionophores⁵⁻¹⁰. X537A causes release of accumulated Ca^{2+} from isolated sarcoplasmic reticulum vesicles and also induces contraction in intact muscle preparations^{8,9}. Both compounds have also been shown to cause secretion of histamine from mast cells, but only in the presence of extracellular calcium⁷. There is no evidence that ionophores are capable of transporting ATP, and although X537A has been found to change the permeability of cells to monovalent cations or amines^{3,5,7}, no such effect has been shown for A23187. In this regard, we also found that the K^{+} ionophore valinomycin did not cause secretion by platelets. Thus the most likely effect of the ionophores in these experiments is to disrupt divalent cation gradients. Since this must involve intracellular ion, the results are consistent with the hypothesis of an internal calcium flux triggering secretion in platelets. We favour calcium as the active ion (ionophores also transport Mg^{2+}) because of the well known role of extracellular calcium in secretion processes². We therefore propose as a tentative model that there is an intracellular store of calcium analogous to sarcoplasmic reticulum in muscle. It is this calcium that triggers secretion. Thrombin causes release of the calcium by a series of steps following binding to the membrane. Ionophores by-pass the thrombin steps (just as they by-pass electrical stimulation in muscle preparations) and directly release the trigger calcium which causes secretion in the normal way.

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RICHARD D. FEINMAN
THOMAS C. DETWILER

Department of Biochemistry,
State University of New York
Downstate Medical Center,
Brooklyn, New York, 11203

Received December 17, 1973; revised February 22, 1974.

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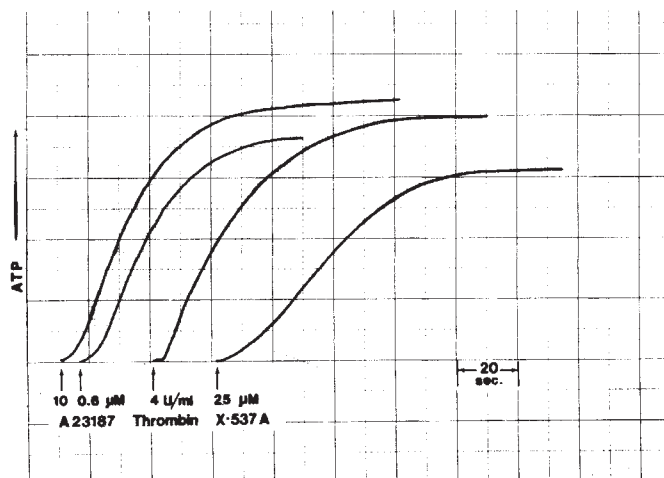


Fig. 1 Thrombin-induced and ionophore-induced secretion of ATP by washed human platelets. The preparation of platelets has been described before and ATP secretion was measured by the firefly luminescence procedure under conditions identical to those in ref. 4. A23187 was added as 5 μl of an ethanol solution and X537A as 5 μl of a dimethylformamide solution. Addition of solvents alone had no effect. Thrombin was purified by the method of Glover and Shaw¹¹ and had a specific activity of 1800 to 2000 U mg^{-1} .

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Urinary excretion of carnitine in progressive muscular dystrophy

ABNORMAL fat metabolism in the muscles of patients with progressive muscular dystrophy is one possible explanation for the aetiology of this genetic disease but the nature of this abnormality, if any, is not known. In dystrophic mice, the skeletal muscles affected are defective in oxidising fatty acids, but can increase synthesis¹⁻³. In this disease, carnitine might be expected to be involved in the impaired fatty acid metabolism, because it is of major importance in the oxidation of long-chain fatty acids as it transports them to the mitochondrial sites where they are oxidised⁴⁻⁶. If there were a carnitine deficiency, fatty acid oxidation would be impaired, resulting in an excessive synthesis of triglyceride from un-oxidised substrate. To examine whether carnitine is involved in the pathogenesis of progressive muscular dystrophy, urinary excretion of carnitine was estimated in patients suffering from generalised muscular atrophy of various aetiologies.

Twenty-four hour samples of urine were collected from 54 patients; 39 with Duchenne-type dystrophy, 4 with facioscapulohumeral dystrophy, 1 with lime-girdle dystrophy and 6 with spinal progressive muscular atrophy and other diseases. As control subjects, 121 healthy men and 101 healthy women, aged from 8 to 45 yr, were studied. Carnitine was isolated from urine by Amberlite-120 (H⁺) ion-exchange column chromatography and was determined by a modification of Friedman's method⁷.

The results are summarised in Table 1. In normal controls, there is an obvious difference between men and women, men usually having a higher excretion rate than women. The results also indicate low carnitine excretion in all 39 boys and men with Duchenne-type dystrophy as compared with the control subjects ($P < 0.001$) and it is evident that carnitine excretion is significantly lower in all patients compared with controls, matched for sex and age. In these patients, no significant correlation was found between carnitine excretion and the severity or the duration of the disease, and the level of serum creatine phosphokinase. Carnitine excretions of the patients with facioscapulohumeral or lime-girdle

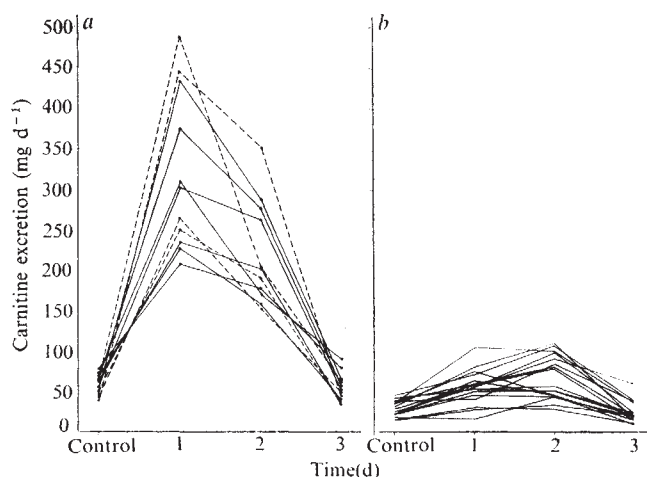


FIG. 1 Changes of urinary excretion of carnitine in patients with Duchenne-type muscular dystrophy and in control subjects before and after injection of synthetic β^{1-24} ACTH-Z. Synthetic β^{1-24} ACTH-Z 1 mg was injected intramuscularly at 0800 h on the first day of the experiment and then urine collections were made consecutively for 3 d. a, Normal control; —, men; ---, women; b, Duchenne-type dystrophy.

dystrophy are within normal range. Six patients with spinal progressive muscular atrophy have high excretions.

Figure 1 illustrates the effect of synthetic β^{1-24} ACTH-Z on urinary excretion of carnitine in the control subjects and in the patients with Duchenne-type dystrophy. ACTH-Z injection stimulated carnitine excretion in the controls, but not in the patients. In other diseases, spinal progressive muscular atrophy and general muscle cramp⁸, ACTH-Z injection did increase urinary excretion to the same extent as that in the controls. ACTH has been known to have some lipolytic activity, but the mechanism whereby it promotes carnitine excretion remains to be elucidated.

Engel and Angelini⁹ demonstrated in a myopathy with excessive lipid that the patient's muscle homogenates had an impaired ability to oxidise long-chain fatty acids because of the low levels of carnitine and that the changes favouring oxidation might occur after addition of carnitine to the homogenates.

The results reported here suggest that a similar deficiency state of carnitine exists in the muscles of Duchenne-type dystrophy but direct evidence is lacking. Further investigations are required to draw any definite conclusion and such studies are now in progress in our laboratory.

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M. MAEBASHI
N. KAWAMURA
K. YOSHINAGA

The Second Department of Internal Medicine,
Tohoku University School of Medicine,
Sendai Japan

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TABLE 1 Urinary excretion of carnitine in healthy control subjects and in patients with neuromuscular diseases

	No. of cases	Carnitine excretion (mg d ⁻¹)					
Normal control male	121	57.7 ± 9.6					
female	101	44.1 ± 2.9					
Duchenne-type dystrophy	39	30.9 ± 3.2					
Facioscapulohumeral dystrophy	4	59.9	60.5	61.8	63.4		
Lime-girdle dystrophy	1	44.7					
Myotonic dystrophy	2	29.2 58.6					
Spinal progressive muscular atrophy	6	63.4	65.5	71.5	92.6	103.0	148.8
Amyotrophic lateral sclerosis	1	71.6					
General muscle cramp ⁸	1	36.8					

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Early hominid ulna from the Omo basin, Ethiopia

A NEARLY complete right ulna (Loc 40-19) was recovered by G. Eck in 1971 in Member E of the Shungura Formation in the lower Omo basin of Southern Ethiopia. It was found in a locality within unit E5 which immediately underlies Tuff F, the K-Ar age for which is 2.04 Myr^{1-4} . This locality has yielded other vertebrate fossils but no further hominid remains in spite of intensive searching. The lower limb of fossil hominids is much better represented than the upper and the remains that do exist are mostly fragmentary. This is the first early hominid forearm bone to be recovered in a state of completeness that will allow locomotor inferences, based on pelvic and lower limb material, to be correlated with good evidence from the upper limb.

The specimen (see Fig. 1) has been damaged both before and after fossilisation and the surface bone has many linear cracks, some of which are more than 1 mm wide and are filled with matrix. The shaft is broken in five places and at the distal three sites it has been possible to reassemble the fragments.

The ulna shaft is long and attenuated. It is curved, convex posteriorly, throughout its length. The sinuous curvature that is seen from below in modern human ulnae is scarcely detectable. The shaft is triangular in section; distally the angles become less well defined. The interosseus border forms an irregular, low crest, which fades out at the level of the bony markings for pronator quadratus. It is unlike the accentuated crest of modern human bones.

The rounded medial border forms the blunt apex of the triangular shaft section. It begins as a low ridge from the medial side of the subcutaneous border of the olecranon and continues distally to a point just proximal to where the interosseus crest is no longer palpable.

The posterior border begins as a crest at the apex of the subcutaneous area of the olecranon. The height of the crest diminishes distally, and it is impalpable in the distal third of the shaft. The border is convex laterally near its proximal end but straightens distally.

The anterior surface of the shaft lies between the medial and interosseus borders. It is damaged at the junction of its proximal and middle thirds; some surface bone is missing and some flakes of bone are depressed below the surface; this area probably included the site of the nutrient foramen. There are also cracks, parallel to the long axis of the shaft, probably produced by antero-posterior crushing of the shaft. The effect of these has been to accentuate the prominence of the medial border. The proximal end of the anterior surface is separated by an irregular ridge of bone from the triangular-shaped area just distal to the radial notch. This rough surfaced area is only very slightly hollowed, in contrast to the marked hollowing for the radial tuberosity in modern human bones.

The postero-medial surface is bounded by the posterior and medial borders. It is curved convexly in its long axis and the distal end follows the spiralling of the shaft.

The postero-lateral surface lies between the interosseus and posterior borders and is the least extensive of the three surfaces. Proximally it is flat from side to side and becomes more convex distally. It is marked by three oblique lines probably related to the attachments of the extensor and abductor muscles. A low ridge, accentuated by linear cracks in the bone, runs along the long axis of this surface, presumably marking the attachment of the deep extensor aponeurosis.

The distal third of the shaft is more cylindrical in section. It is distinguished by a prominent curved crest of bone which runs between the anterior and postero-medial aspects, marking the origin of the pronator quadratus.

The distal end of the specimen is expanded. It includes most of the head, but the posterior aspect of the shaft and the styloid process are missing. The head bears a convex articular surface on its antero-lateral side for articulation with the radius, and a much smaller, less distinct, distally directed articular surface. These surfaces, seen from below, are crescentic.

The proximal end of the ulna bears two processes, the coronoid and olecranon, and two articular areas, the trochlear and radial notches. Only the olecranon process has escaped significant damage.

The proximal surface of the olecranon is marked, from above downwards, by synovial reflection, the triceps bursa and the triceps insertion. A crest separates the olecranon from the rough triangular, subcutaneous area of the proximal end of the ulnar shaft. The subcutaneous area is in line with the posterior border of the shaft; viewed from the side, it does not project posteriorly as it does in some non-hominid ulnae. At the medial border of the olecranon process is a pronounced tubercle for the origin of flexor carpi ulnaris and for the insertion of the posterior fibres of the medial ligament of the elbow point. Distal to the tubercle is a strongly concave area for the origin of flexor digitorum

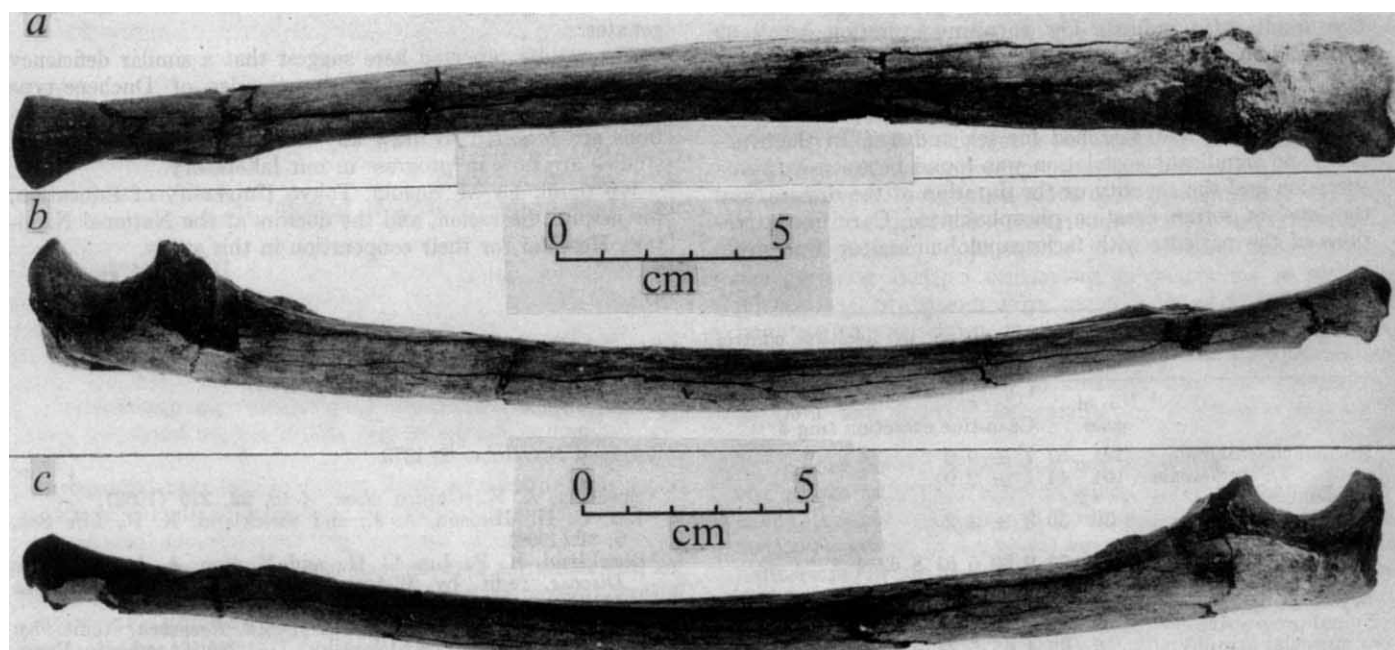


FIG. 1 a, Superior view of Omo ulna (Loc 40-19); b, lateral view; c, medial view.

TABLE 1 Length and curvature indices of Omo specimen and of ulnae of other higher primates

Specimens		<i>Homo</i> (=45)	<i>Pan</i> (=11)	<i>Gorilla</i> (=14)	<i>Pongo</i> (=10)	<i>Hyalobates</i> (=3)	Omo (Loc 40-19)
Maximum length	Mean	250	291	346	367	249	315
	s.d.	19.0	14.8	38.5	24.8	23.0	—
	s.e.	2.8	4.6	10.3	8.0	13.5	—
Relative length index	Mean	12.0	14.5	12.5	16.0	24.9	13.0
	s.d.	1.3	2.5	6.6	1.9	2.4	—
	s.e.	0.2	0.8	1.8	0.6	1.4	—
Curvature index	Mean	7.2	11.0	11.4	8.1	3.4	10.5
	s.d.	1.1	1.4	1.6	1.1	0.2	—
	s.e.	0.2	0.4	0.4	0.3	0.1	—

profundus. The shaft does not project proximal to the olecranon process as it does in baboon ulnae.

The coronoid process is extensively damaged along its margins. Its anterior surface, and the adjacent part of the shaft, are occupied by the ulnar tuberosity. The antero-medial side of the tuberosity is marked by a pronounced fossa for the insertion of brachialis. The lateral edge of the fossa is elevated and may be for a head of flexor pollicis longus.

The trochlear notch lies between the two processes, and is formed by two articular surfaces separated by a roughened area of bone. The proximal of the two articular surfaces is divided by a flattened central area into two nearly equal, slightly concave, areas for the flanges of the trochlea of the humerus. The distal articular surface on the coronoid process is incomplete and is divided by a ridge into a larger medial and smaller lateral surface. The lateral surface meets, at an angle, the laterally facing, concave, radial notch. Both the notch and the supinator crest, that leads distally from it, are extensively damaged.

Preliminary examination and comparisons indicate that this new fossil resembles in many respects modern human ulnae, but some features depart significantly from the modern human pattern. Morphological assessment has been supplemented by measuring some 20 parameters. The same measurements have been taken on samples of extant higher primates. These samples are small in some instances but prove adequate for this preliminary study. Features divergent from the modern human condition include:

(1) **Length of the shaft.** In the Hominoidea relative elongation of the forelimb is generally associated with suspensory postures, and this is demonstrated by the intermembral index. That this elongation is largely due to lengthening of the forearm can be demonstrated by the brachial index⁵. The maximum length of this fossil ulna and ulnae of other higher primates are given in Table 1. Using the *t* test, the probability that the fossil specimen could be included in the *Homo* sample is low ($P = <0.005$). Limb lengths are only useful when related to total body size, but from indirect evidence there is no reason to suppose that fossil hominids were taller than the modern *Homo sapiens* populations from which our sample was taken. The maximum length therefore suggests an elongated forearm for this particular fossil hominid. We have attempted to measure relative elongation of an isolated ulna by using the index

Maximum shaft length (Martin⁶ (1))

Olecranon to coronoid distance (Martin (7))

The results (see Table 1) show that relative length can be related to locomotor habit—suspensory forms have high values. There is so much overlap, however, between the ranges of *Gorilla* and *Homo* that the significance of the fossil's value is difficult to assess.

(2) **Dorso-ventral curvature of the shaft.** The values for the curvature of the ulnar shaft, expressed as an index (Martin (4)), in which highly curved bones have high values, are also given in Table 1. In general, curvature is more marked in knuckle walkers than in suspensory forms. *Homo* occupies an intermediate position. The curvature seen in the African apes is presumably a response of the ulnar shaft to stresses

peculiarly associated with knuckle walking, for *Papio*, with a digitigrade forelimb stance, has a straight ulna. The value of the index for this fossil specimen falls outside the human range and within that of the knuckle walkers; the probability levels are $P = <0.005$ and $P = 0.3$ respectively.

(3) **Cross-sectional profile of the ulna shaft.** Human ulnae generally have a prominent interosseus border which suggests that stresses on the interosseus membrane have dominated the final shape of the modern human ulnar shaft. A well marked bony shelf is uncommon in other hominoids and is absent in this fossil. Its absence, combined with the dorso-ventral flattening of the shaft, may indicate significantly different patterns of stress than those seen in modern human ulnae. It is possible that these differences are associated with changes in the relative bulk of forearm flexor and extensor muscles comparable with the changes which Tuttle⁷ has demonstrated between knuckle walkers and humans.

(4) **Pattern of muscle origins and insertions.** The pattern of muscle markings in the fossil broadly parallels that found in modern human ulnae, though two differences may be significant. The first is the flatness and lack of buttressing of the ulnar tuberosity. The second is the weak development of the supinator crest compared with that of modern human ulnae. Further specimens may be required to determine whether these distinctions are merely the result of intra-group variability, which is substantial in man and other higher primates, or whether they reflect genuine differences in the development of the brachialis and supinator muscles in this hominid.

(5) **Shape of the ulna head.** Generally, the crescentic articular surface of the ulna head is more convex in modern human ulnae than it is in this fossil. Even if this difference is significant, its functional implications are not clear.

Overall, the functional implications of these differences from modern human ulnae point to a relatively elongated forearm and some morphological adaptations in the fossil not unlike those seen in modern knuckle walkers.

Unfortunately the ulna is still one of the least well represented parts of the skeleton among the early Hominidae. Three specimens are known for certain. Two are proximal fragments, one from Kromdraai (TM 1517) and one from East Rudolf (KNM-ER 1500); both are smaller than the Omo fossil. Their fragmentary state and the high intra-group and low between-group variability in ulnar morphology in higher primates strictly limits their usefulness.

OH, 36 ulna from Bed II Olduvai Gorge, Tanzania is complete apart from its distal end. As judged by the positions of structures that are common to both ulnae its relative length is shorter by approximately 5% than the Omo specimen and its maximum length is approximately the same as the Omo ulna. It is also more curved. The significance of any differences between the two fossils must await the detailed assessment of both specimens.

It is clear that the affinities of the Omo fossil are with the Hominidae. Mandibular and dental remains of Hominidae have been found at 11 localities within Member E of the

Shungura Formation. All, or nearly all, of this material seems to represent a robust australopithecine, but there are still no known associations of cranial and dental and postcranial parts of this hominid in most of the Omo succession. It is difficult therefore to determine the taxonomic affinities of this specimen. It seems to us that any such judgment must rely strongly on morphological features, and to a lesser extent on the functional capabilities inferred from them. These can then be related to existing hypotheses about the locomotor patterns of early hominids.

There is increasing evidence from other early hominid postcranial remains, from South and East Africa, that the morphology of robust australopithecines differed significantly from that of *Homo*⁸⁻¹². This is now shown more clearly by material, recently announced, from East Rudolf, which is very like *Homo* and carries this dichotomy probably back beyond 2.6 million years¹³. Consequently, in view of the differences between the Omo fossil specimen and modern human ulnae, we feel that this ulna is probably that of a robust australopithecine and should be referred to *Australopithecus boisei*.

The relative elongation and the presence of certain features suggestive of a role in body support in this fossil do not necessarily mean that this creature used habitual suspensory postures or that it knuckle walked. Both these suggestions have been put forward, the first by Robinson¹², and the second by R. E. F. Leakey¹⁴ in connection with a hominid humerus (KNM-ER 739) from East Rudolf. Tuttle, and others before him, have cogently argued that knuckle walking is a specialised means of fore-limb propulsion and support developed as a consequence of an arboreal form subsequently adapting to a terrestrial life. The morphological and functional evidence seen in this specimen is insufficient to infer such a specialised locomotor adaptation for this hominid.

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F. CLARK HOWELL

Department of Anthropology,
University of California
Berkeley, California 94720

B. A. WOOD

Department of Anatomy,
Charing Cross Hospital Medical School,
London W6 8RF

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The effect of prostaglandin F_{2α} on ovarian blood flow and corpora lutea regression in the rabbit

IN many species luteolysis is dependent upon the presence of the uterus and an intact utero-ovarian vasculature. The occurrence of prostaglandin F_{2α} (PGF_{2α}) in uterine tissue, its specific vasoconstrictor properties and its ability to induce luteolysis exogenously, led to the hypothesis that its release by the uterus might cause luteolysis by constricting the uterine and utero-ovarian veins and reducing ovarian blood flow¹. The observation that total ovarian venous outflow fell during infusions of PGF_{2α} in rats and rabbits (after laparotomy and ovarian vein cannulation) supported this hypothesis. A subsequent investigation using similar methods failed to confirm this in rats². Experiments in sheep with ovaries and uterus transplanted *in toto* to the neck produced no unequivocal evidence for a drop in ovarian blood flow following doses of PGF_{2α} producing luteolysis, at least until oestrus ensued³. With increasing doses however, an immediate fall in ovarian blood flow occurred⁴.

In rabbits from mid gestation to near term, the corpora lutea receives about 80% of the total ovarian arterial flow⁵. It is therefore possible that a substantial decrease in corpora luteal blood flow could be effected by regional redistribution of blood within the ovary without affecting the total flow. Novy⁶ used radioactive microspheres to measure ovarian blood flow distribution in pseudopregnant rabbits and observed a 25% reduction in luteal flow on injection of PGF_{2α} intravenously. No significant alteration in total arterial ovarian blood flow occurred. In three sheep, which were not pregnant, corpora luteal blood flow, measured with radioactive microspheres, fell within 5.5 h from the start of a PGF_{2α} infusion

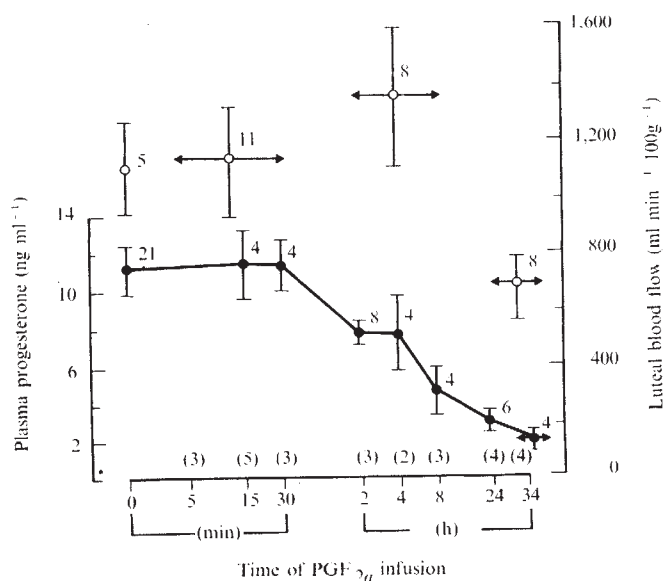


FIG. 1 Effect of PGF_{2α} infusion on peripheral plasma progesterone (●) and corpora luteal blood flow (○). Mean values with standard error and the number of observations are shown. The number of rabbits injected with microspheres at each time from the start of infusion is shown in brackets. Corpora luteal blood flows at time zero were determined by Abdul-Karim and Bruce⁵.

TABLE 1 Ovarian blood flows (ml per min per 100 g (mean $\pm$ s.e.)) after 250 μ g h⁻¹ PGF_{2 α} given intra-arterially

Infusion time	N	Corpus luteum	Stroma	Total ovary
zero	5	1155 $\pm$ 212	127 $\pm$ 9	435 $\pm$ 59
5-30 min	11	1171 $\pm$ 196	674 $\pm$ 94*	809 $\pm$ 123
2-8 h	8	1360 $\pm$ 245	472 $\pm$ 62*	725 $\pm$ 127
24 h and delivery	8	689 $\pm$ 109	202 $\pm$ 42	327 $\pm$ 59

* significantly greater than zero time control ($P < 0.005$; t test)

into the uterine vein⁷. Luteolysis itself may affect the rate and distribution of ovarian blood flow. We therefore investigated the effect of PGF_{2 α} on the chronological order of changes in ovarian blood flow and its distribution to test the hypothesis that alterations in these parameters may precede induced luteolysis.

Twenty-nine pregnant rabbits were used. The day of mating was denoted as day 0 of gestation. PGF_{2 α} (5 ml h⁻¹) in saline solution was infused in 27 and saline alone in 2 rabbits through an aortic catheter introduced through a femoral artery, the tip being 4 cm above the origin of the ovarian arteries. The dose of PGF_{2 α} was 250 μ g h⁻¹ which induces labour in unanaesthetised rabbits⁸. Anaesthesia was induced and maintained by an initial dose of 30 mg kg⁻¹ sodium pentobarbitone and further small intravenous injections until the injection of microspheres as previously described⁹. When the infusion of prostaglandins lasted more than 8 h, sodium thiopentone anaesthesia was used for the initial placement of the infusion catheter; the rabbits were then allowed to recover and were anaesthetised with sodium pentobarbitone about 1 h before the microsphere injection. In preliminary trials, these procedures had little effect on ovarian blood flow. About 100,000 microspheres 25 μ m in diameter labelled with ⁴⁶Sc were injected into the left ventricle over 30 s, at various times after the start of the infusion (Fig. 1). Arterial plasma progesterone concentrations were measured before and during infusion with a radioimmunological method based on that of Thorneycroft and Stone¹⁰ using petroleum ether (Mallinkrodt 30°-60°C Nanograde) instead of diethyl ether. The blood flow measurements were made on day 28 of gestation except in the two rabbits infused with saline (day 29 and 30). After the microspheres were injected, the rabbit was killed and the position of the infusion catheter was checked. The ovaries were separated into luteal and stromal components, weighed and measured for radioactivity to determine blood flows⁹.

Arterial blood pressure did not alter significantly during the infusion of PGF_{2 α} , saline or during microsphere administration. There was a significant fall ($P < 0.01$; t test) in peripheral plasma progesterone within 2 h of the start of PGF_{2 α} infusion (Fig. 1). This fall continued to delivery. The corpora luteal arterial blood flows were combined into three groups, those measured before the fall in plasma progesterone (5, 15 and 30 min after the start of PGF_{2 α} infusion), those measured after a significant decline in plasma progesterone (2, 4 and 8 h) and those measured at 24 h or at delivery (Fig. 1). Blood flows in five control rabbits which were not infused⁵ are also shown. There was no evidence of a decline in corpora luteal blood flow for at least 8 h after the start of PGF_{2 α} infusion.

Even at 24 h, the mean flow was 825 $\pm$ 172 (s.e.) ml per min per 100 g ($n = 4$) or 71% of the control value. A substantial fall, to 552 $\pm$ 115 ml per min per 100 g was observed only in the four rabbits infused with PGF_{2 α} to delivery. No difference was noted between flow rates in two rabbits receiving microspheres at delivery and one each at 0.5 and 4 h after delivery. Corpora luteal blood flow was 1,590 and 817 ml per min per 100 g in the rabbits infused

with saline at 30 and 60 min following delivery, after infusions for 62 and 100 h respectively. At this time plasma progesterone was low (3.1 and 4.4 ng ml⁻¹). Table 1 shows corpora luteal, stromal and total ovarian arterial blood flows. Prostaglandin F_{2 α} infusion had a rapid large effect on ovarian stromal flow. Five minutes after the start of infusion flow rose from a control value⁵ of 127 $\pm$ 9 ($n = 5$) to 987 $\pm$ 209 (range 609-1330) ml per min per 100 g ($n = 3$). Stromal flow remained high for at least 8 h of infusion but returned to near normal at 24 h. When divided in the same way as corpora luteal blood flows, stromal flow was significantly greater between 5 min and 8 h infusion of PGF_{2 α} but not at 24 h or delivery. Changes in total ovarian blood flows and corpora luteal blood flows were not statistically significant.

We conclude that in the rabbit luteolysis induced by PGF_{2 α} is not preceded by a biologically significant reduction in the arterial blood flow to the corpora lutea. The mechanism of action lies elsewhere. The rise in ovarian stromal blood flow, also observed by Novy in pseudopregnant rabbits⁶ is intriguing. In our experiments it was associated with a transitory decrease in placental blood flow and an increase in vaginal and cervical flow (N.W.B. and K.H., unpublished). Whether the circulatory changes in the stroma act indirectly to initiate luteolysis remains speculative, but a direct effect of PGF_{2 α} on corpora luteal tissue must be considered.

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NEVILLE W. BRUCE*

Nuffield Institute for Medical Research,
Headley Way,
Oxford

KEITH HILLIER

Nuffield Department of Obstetrics and Gynaecology
University of Oxford,
John Radcliffe Hospital
Oxford

Received January 11, 1974.

* Permanent address: Department of Anatomy, University of Western Australia, Nedlands 6009, Western Australia.

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Placental transfer of asbestos

ASBESTOS fibres have been reported to penetrate the walls of the stomach and intestine of animals^{1,2}. Earlier work at this institution showed that asbestos fibres can pass through the walls of the digestive tract and travel throughout the body^{3,4}. Additional work with neutron-activated asbestos also indicated that asbestos could cross the placenta but definite confirmation of this was not obtained⁴. Here we show, by electron microscopy, that asbestos fibres can cross the

TABLE 1 Asbestos fibres in foetal liver and lungs from pregnant rats injected with asbestos

Rat No.	Total asbestos injected intravenously (mg)	Number of foetuses Experiment 1	Foetal liver fibres g ⁻¹ (× 10 ⁶)	Foetal lung fibres g ⁻¹ (× 10 ⁶)
1	0	13	0	0
2	0	11	0	0
3	0	12	1.38	1.52
4	0	11	0	0.23
5	9*	11	2.83	1.00
6	9*	14	2.93	1.25
7	12	12	27.03	139.97
8	12	13	18.34	1.26
Experiment 2				
9	0	13	0	0
10	0	16	0	0
11	0	12	0	0
12	0	16	0.45	0
13	4	12	7.59	0.14
14	4	10	0	4.32
15	4	7	3.95	0.38
16	4	8	1.38	1.38
17	10	9	2.67	1.33
18	10	14	100.12	2.90
19	10	8	39.32	0
20	10	8	5.65	3.91

* Young were born about 2 h after the third injection but were killed immediately.

placenta but that the extent to which this occurs is highly variable.

The same chrysotile asbestos preparation used in earlier work^{3,4} containing approximately 10×10 fibres μg^{-1} was injected into the femoral vein of 300 g pregnant Wistar rats at levels of 1 to 3 mg (1 mg ml⁻¹ water) at 2 d intervals beginning on the 10th to 14th day of gestation. The foetuses were removed by Caesarean section on the day before parturition to ensure that they did not inhale any asbestos fibres. To prevent cross contamination of the blood of the mother with the tissues of the foetuses the rats were killed by inhalation of ether and the foetuses within their intact amniotic sacs were removed from the uterus. Upon removal of the amniotic membranes the foetuses were opened and the lungs and livers saved for asbestos analysis. The livers from two foetuses (about 0.2 g) and the lungs from three (about 0.4 g) from each rat were analysed by previously described techniques⁴.

In the first experiment four rats received 3 mg doses of asbestos. Two of these received 12 mg before they were sacrificed, but the other two bore young about 2 to 3 h after the third dose, apparently initiated by the stress of the injection. Preliminary experiments indicated that higher doses were usually fatal with the rats showing difficulty in breathing. This may be related to the large amount of intravenously injected asbestos which initially lodges in the lungs⁴. Extremely high values were recorded in the foetal liver and lungs from rat 7 and foetal liver from rat 8 (Table 1) but the level of asbestos fibres in the foetal tissues from rats 5 and 6 were close to the higher values in the control rats. For this reason a second experiment (Table 1, experiment 2) was conducted in which four rats again served as controls, four rats received four 1 mg injections of asbestos and four rats received five 2 mg injections.

The control values were quite low in this experiment with all but one falling below a detection level of one fibre on 10 electron microscope grid squares equivalent to 0.45×10^6 fibres g⁻¹ in liver or about half this amount in lungs. Foetal liver and lung samples from rats receiving asbestos were again quite variable with extremely high values recorded in the livers of several rats. Such variable results are very difficult to analyse statistically but the high values are quite important in that they could result from a breakthrough of asbestos fibres in the placentas of some foetuses. A large inflow of asbestos into the blood of one foetus would probably

raise the asbestos level in all its organs. This is not evident from the present data as the livers and lungs analysed were selected at random and could have come from different foetuses in the same uterus. It is possible that the extremely high values in livers of foetuses of rats 18 and 19 are the result of one or more particularly large asbestos fibres breaking through an opening between the maternal and foetal circulation in the placenta allowing many smaller fibres to follow in its wake. It is also possible that a large number of small fibres piercing the placental membrane could destroy one or more vital cells resulting in a disruption of the membrane. If this were the case and there was an intermingling of the two circulations this could suggest an additional hazard of asbestos fibres.

We conclude that the number of very high values recorded in the rats injected with asbestos compared with very low levels in the controls in the two experiments adds considerable evidence to the theory that asbestos fibres can cross the placenta. The way in which this occurs, however, by individual penetration, by a mass breakthrough or both has yet to be determined.

H. M. CUNNINGHAM
R. D. PONTEFRACCT

Health Protection Branch,
Department of Health and Welfare,
Ottawa, Canada

Received December 31, 1973.

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Magnetic field perception by electroreceptors in Black Sea skates

THE perception of the Earth's magnetic field by vertebrates for orientation purposes has been discussed for over a century¹⁻⁴. New approaches to this, still unsolved, problem were made after the lateral line of some fish species was shown to contain electroreceptor structures^{5,6}. The great sensitivity of these structures to electrical stimulation suggested that they may be an apparatus for detection of a

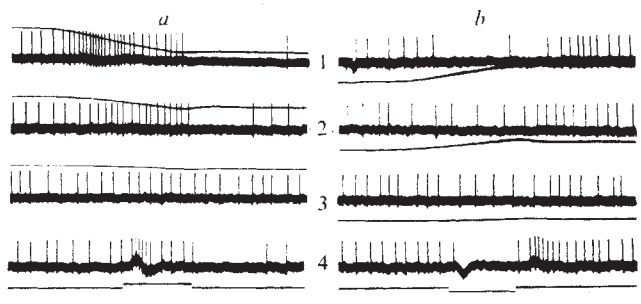


FIG. 1 Responses of the neurone in the area acoustico-lateralis of the medulla oblongata to magnetic stimuli of various intensities and opposite directions and to electrical stimuli of opposite polarities. Magnetic stimulus: 1, 80 gauss s^{-1} ; 2, 36 gauss s^{-1} ; 3, 9 gauss s^{-1} . a, South direction; b, north direction. 4, Responses to electrical stimulus of 3×10^{-9} A mm^{-2} . a, Cathodal stimulation; b, anodal stimulation. Time bar is 500 ms. Record of magnetic induction is 80 gs.

changing magnetic flux and thus have a role in fish orientation by means of the Earth's magnetic field⁷⁻⁹. The possibility of magnetic perception by animals having the electroreceptor apparatus, has been shown in behavioural experiments^{6,10}. No direct neurophysiological data on this problem are available so far.

To investigate this problem we carried out experiments on the Black Sea skates (*Trigon pastinaca*), which have a well developed electroreceptor apparatus of ampullae of Lorenzini^{10,11}. In our experiments, the single unit activity in the area acoustico-lateralis of the medulla oblongata was recorded with glass microelectrodes. Responses to electrical stimulation, as well as to the changing magnetic flux through the fish, were studied. Electrical stimuli were applied through the pair of silver electrodes placed on the skin of the fish. Magnetic stimuli were caused by a linearly increasing current of different characteristics, passed through the winding of electromagnet.

Our experiments showed that the changing magnetic field penetrating the fish evoked the response of neurones in the area acoustico-lateralis (Fig. 1), whereas the constant magnetic field failed to influence the electroreceptor system. The characteristic of the response to the changing magnetic field is dependent on the direction of the field; the intensity of the reaction is due to the rate of change of the magnetic field. The responses to magnetic stimulation could be recorded only in these neurones connected to the electroreceptor system which could easily be recognised by the responses of the neurones to electrical stimulation (current density thresholds were in the range of 10^{-9} to 10^{-10} A mm^{-2}).

The smallest change in the magnetic field evoking a neuronal response was found to be 2 gauss s^{-1} , obtained on a neurone showing no spontaneous activity. Apparently, this value could be reduced by improving the functional state of the animal.

The comparison between the magnetic flux change observed in experimental conditions and that which occurs during natural fish movements (both linear and turning) shows the possibility that the Earth's magnetic field may be perceived by electroreceptor apparatus of ampullae of Lorenzini.

H. R. BROWN
G. N. ANDRIANOV
O. B. ILYINSKY

Laboratory of the General Physiology
of Perception,
Pavlov Institute of Physiology,
Academy of Sciences of the USSR,
Makarov emb.
6, Leningrad 199164, USSR

Received November 1, 1973; revised February 11, 1974.

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Is polymorphism in two-spot ladybird an example of non-industrial melanism?

THE polymorphism exhibited by the two-spot ladybird, *Adalia bipunctata* Linn., in which specimens have a red ground colour and black spots (the typical morphs) or a black ground colour with red spots (the melanic morphs) is a well known phenomenon. The reasons for the existence of this polymorphism, however, are not well understood. Analogy to the situation in some melanic Lepidoptera, such as *Biston betularia* Linn.^{1,2}, is unacceptable as it would seem that selective predation by birds upon the morphs which blend less well with their surroundings would not operate upon *A. bipunctata*. Ladybirds are distasteful, have a distinctive scent, are warningly coloured and therefore are avoided by vertebrate predators^{3,4}.

Studies of the frequencies of the morphs of *A. bipunctata* in Great Britain have been undertaken by Creed⁵⁻⁷. He postulates that the distribution of melanics is related to industrial pollution and speculates that some, as yet unidentified, component of the polluted atmosphere is toxic to the typical morph. This explanation is not altogether satisfactory, however, for example, melanics seem to be non-existent in central and eastern London, and were present at a very low frequency before smoke control regulations were introduced^{8,9}. Furthermore, there are high frequencies of melanics in rural areas of north-west England. Specimens of the melanic morph were collected as early as 1696; 150 yr before the earliest records of industrial melanism in the Lepidoptera¹⁰. For these reasons, together with the absence of an identified selective toxin, we re-examined the alternative hypothesis of Lusi¹¹ that a climatic factor, in particular solar radiation, may determine the melanic frequency.

Differences have been recorded in the temperature of small brass objects painted black or white and exposed to sunlight¹², while melanism related to seasonal temperature variations has been demonstrated in an aphid¹³. Measurements of the metabolic rates of samples of *A. bipunctata* (containing both typical and melanic morphs) showed that in the dark oxygen consumption rose from 175 μl per g live weight per h at 10° C to 296 μl g^{-1} at 15° C, 721 μl g^{-1} at 20° C and 985 μl g^{-1} at 25° C. Thus, even a small rise in temperature causes an increase in the metabolic rate of the insect. Pairs of melanic and typical insects were enclosed in tubes and chilled in ice for 30 min. Their movements were then recorded over a period of 12 min immediately following their introduction into an arena containing a 25 mm grid. Measurements of their activity were made at two temperatures, 5° C

TABLE 1 The activity of melanic and non-melanic forms of *A. bipunctata* measured as the number of 25 mm squares entered in 12 min

Temperature (°C)	Melanic	Non-melanic
5.0	33.4 $\pm$ 7.0	8.8 $\pm$ 2.04
7.5	67.0 $\pm$ 8.17	51.0 $\pm$ 9.23

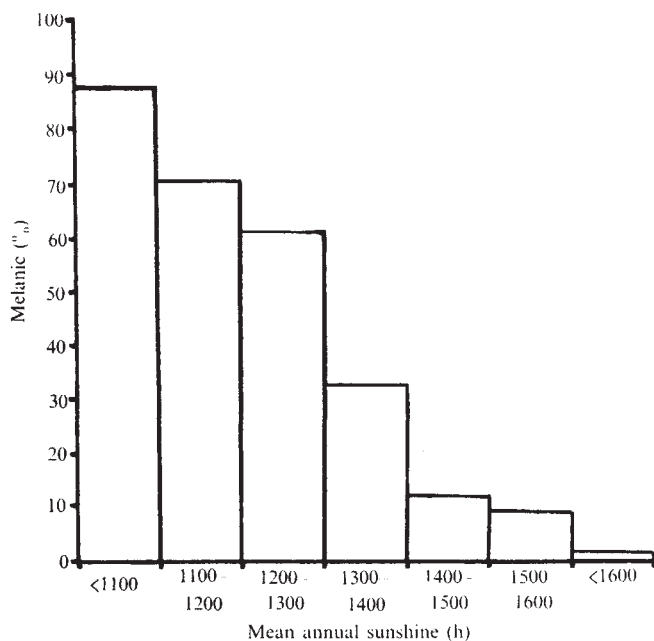


Fig. 1 Percentage of melanics in each 'sunshine class'.

and 7.5° C. Illumination was provided by a 100 W lamp suspended above the arena. The melanic insects showed greater activity at both temperatures (Table 1).

In view of the effect of illumination on activity, we believe that a lack of sunshine is the principle factor in the selection of the melanic morphs. The higher level of activity attainable by the melanic morphs will give them considerable competitive advantages in the search for food. This is especially true in areas where sunshine levels are low enough to reduce the period of activity to a point where it becomes a critical factor in the life of the insects. We have found that related species require warm, sunny conditions for mating and egg laying¹⁴ and the same may be true for *A. bipunctata*. Thus, in localities with low sunshine levels, the mating and egg laying activity of the melanic morphs may greatly exceed that of the typical morphs. Smoke pollution may be expected to be a secondary factor in the determination of melanic frequencies as it will reduce the incident solar radiation in some areas to the critical level.

We have estimated the mean annual sunshine hours for the sites listed by Creed^{6,7} from records for the years 1921-50 (ref. 15). The sites were then placed in 'sunshine classes' representing 100 h increments in the annual total. The % of melanics in each sunshine class was calculated from the numbers of the different morphs at each site. The results (Fig. 1) show a very close relationship between melanic frequency and annual sunshine hours. A correlation between melanic frequency and sunshine level at each site shows a highly significant negative correlation ($r = -0.59$, $P < 0.001$).

It seems that a re-examination of the theory of industrial melanism, as applied to *A. bipunctata*, is justified.

B. R. BENHAM

Science Department,
Bolton Institute of Technology,
Bolton, Lancashire

D. LONSDALE

Cryptogamic Botany Laboratories,
The University, Manchester

J. MUGGLETON

Department of Zoology,
The University, Manchester

Received December 12, 1973; revised January 24, 1974.

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Changes in the plankton community of the Western English Channel

MARINE biologists and oceanographers will probably be familiar with the story of the biological and chemical changes that occurred in the Channel waters off Plymouth during the 1930s: a summary of how the plankton community altered, the numbers of young fish declined, adult demersal fish became scarcer and herring were replaced by pilchard can be found in many textbooks. The most striking feature, and one of the first to be detected, was the changeover from a plankton community characterised by the chaetognath *Sagitta elegans* and associated 'plankton indicators' to a much poorer community characterised by *Sagitta setosa*¹⁻³.

Since 1965, signs of a reverse change in biological conditions have been evident, and reports on the striking improvement in the numbers of young fish since then have already been published^{4,5}. By 1970 the numbers of the copepod *Calanus* during the spring peak of zooplankton (May and June) were equal to those found in 1930 (ref. 6). From 1933 to 1971, however, *Sagitta setosa* remained the dominant chaetognath in the weekly samples taken off Plymouth; *S. elegans* was either completely absent or occasionally present in very small numbers^{3,6-15}, although in

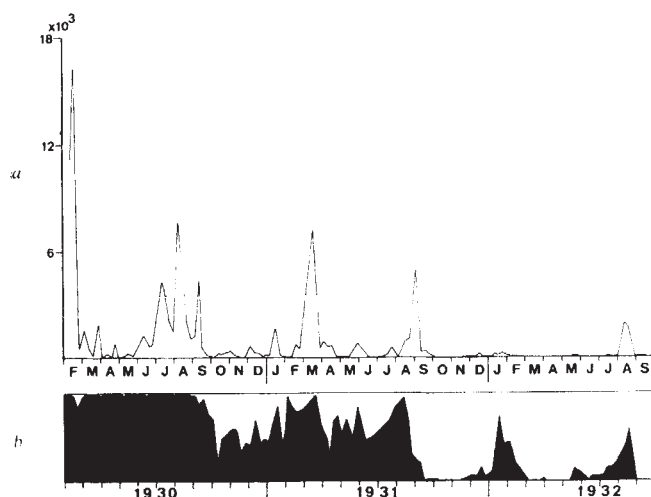


Fig. 1 *a*, Seasonal changes in the abundance of *S. elegans* (as numbers per standard haul of a 2 m net at station L5) in 1930-32, and, *b*, the proportion of *S. elegans* in the mixed population of *S. elegans* and *S. setosa* (redrawn from Russell).

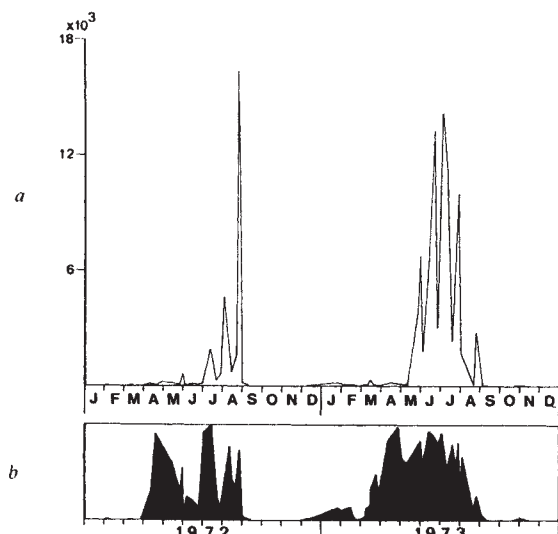


FIG. 2 a, Seasonal changes in the abundance of *S. elegans* (as numbers per standard haul of a 2 m net at station L5) in 1972-73; b, the proportion of *S. elegans* in the mixed population of *S. elegans* and *S. setosa*.

the later years after 1958 it was slightly more common in mid-channel and to the west^{6,16}.

It is therefore of great interest to report that in 1972, for the first time since 1932, *Sagitta elegans* was found in considerable numbers off Plymouth, even at inshore stations, and that in 1973 it was even more abundant and was the dominant species at the Eddystone station (L5) for 5 months. Over the period May to August inclusive, *S. elegans* was present in numbers equal to or greater than those recorded in the 1930s (Figs 1 and 2). The agreement between the results of weekly sampling in 1930-32 and more recent results is not complete, especially with regard to the proportions of the two species in certain of the months. The recent samples are collected with a net of very slightly narrower meshes, which retains more of the smaller sizes of chaetognaths: the total numbers of *S. elegans* (Fig. 1a and Fig. 2a) are somewhat greater, and their proportion in the combined chaetognath population (Fig. 1b and Fig. 2b) somewhat less than in the earlier period. Although the 1972 and 1973 samples did not show the spring peak of *S. elegans* found in 1930 and 1931, there were clear signs of a recurrence of this peak in 1974 and therefore the seasonal trends in abundance agree quite well.

That this return of *S. elegans* is not an isolated change is shown by the occurrence of the medusa *Aglantha* and the siphonophore *Nanomia* during the recent summers when *S. elegans* was abundant. Furthermore, in winter the euphausiid crustaceans *Meganctiphanes* and *Thysanoessa* have been taken in night samples to the west and south-west of the Eddystone. A combination of all these north-western plankton indicators⁷ with large numbers of *Sagitta elegans* has not been observed off Plymouth in any year since 1930 (ref. 17). Other indicators, such as the pteropod mollusc *Spiratella retroversa* and the larvae of the starfish *Luidia sarsi* have been abundant at certain times in the past few years, while warmer water species have become scarcer.

The recent evidence thus reinforces the view that the biological reversal reported in 1971 (ref. 6) was not a minor fluctuation but part of a much longer trend. It also seems that the order of the changes is being repeated inversely. Thus, in 1930-35 the replacement of *S. elegans* by *S. setosa* was followed by a decline in numbers of young fish and other macroplankton, and later by the appearance of large numbers of eggs of pilchard during the summer. In the more recent period a reduction in the summer pilchard eggs has been experienced since 1963, the young

fish have returned in great numbers since 1966, and the numbers of the copepod *Calanus* came back to their former peak in 1970: only now, in 1973 is there a real abundance of *S. elegans*. An analysis of the corresponding hydrographic changes during the past ten years is being made by my colleague, E. I. Butler, but there seems no reason to doubt the suggested overall link with climatic fluctuation: some of the temperatures in the deeper waters below the thermocline in the past 2 yr have been the lowest recorded since the 1920s.

It could be suggested that what we have been observing off Plymouth since the 1920s is part of a cyclic fluctuation, with a peak-to-peak frequency of the order of 40 to 50 yr. *Sagitta elegans* seems to have been the first of the plankton species to show the change and the last to return, suggesting that in some way it could be more 'sensitive' to the causes of the fluctuation. Therefore a continued monitoring of this species and its associated 'indicators' may be helpful in detecting future changes in the ecosystem of the western Channel, whether naturally induced or otherwise.

A. J. SOUTHWARD

Marine Biological Association of the United Kingdom,
The Laboratory,
Citadel Hill,
Plymouth, PL1 2PB

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Microorganisms participate in the construction of manganese nodules

THE geographical distribution and formation of manganese nodules have inspired investigation and speculation for more than a 100 years (for example, refs 1 and 2). Although photographs have revealed that some organisms attach themselves to Mn nodules on the deep seafloor (for example, ref. 3), these were apparently epiphytes, not contributing to the formation of the nodules. I now wish to suggest, on the basis of an ultrastructural examination of Mn nodules, that several groups of organisms are active in their construction.

I have found that: (a) numerous shelter-building organisms reside on the surface of deep-sea Mn nodules; (b) benthic foraminifera of the genus *Saccorhiza* attach Mn micronodules to the surfaces of macronodules as part of their tube-building activities; (c) some tubes of *Saccorhiza* and other benthic organisms originally constructed on nodule surfaces are buried and preserved (not replaced) within the interior of nodules; (d) other organisms on nodule surfaces build structures that contain large amounts of Fe and Mn compounds; (e) biologically derived structures cover large portions of surfaces of all the seventy-one nodules from the Pacific Ocean basin that I have examined so far.

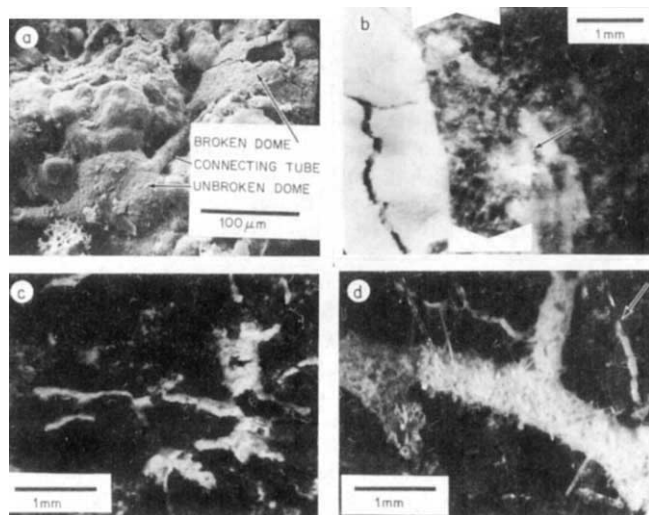


FIG. 1 Micrographs of nodule surface structures (oblique reflected light was used on all photographs except (a) which is from scanning electron microscopy). Small tube and dome structures (a) have very little contrast with the general nodule background. These structures occur in patches, or active regions, and are internally clean and empty. Structures near the borders of active regions are filled with a secondary matrix. One dome is ruptured and vacant while a second dome is intact. Massive, white, spined body is presumably a sponge (b) across which is a trail (marked) rich in Mn. The trail clearly proceeds from the nodule surface across the white mass and back onto the nodule surface. The arrow shows where a portion of the trail was removed to confirm that it is not the nodule surface protruding through the white mass. The organism responsible for this trail may be important in modifying the existing nodule surface by depositing or redistributing sheets of manganese oxides. Broken, partially infilled and partially covered *Saccorhiza* tubes (c) have some infilling material that appears to be pre-existing micro-nodules cemented in place by the tube builder while some appears to have been deposited in place as smaller tube and dome structures. This should be compared with the undamaged tube in (d). The smaller white tube (d) should be compared with Fig. 2.

Most organisms potentially important in the origin and development of nodules are microscopic (<0.5 mm across). They create fragile primary structures that are usually destroyed by any but the most careful collection process, partly explaining the lack of study of the organisms until modern sampling devices made possible recovery of undisturbed nodules.

The structures discussed here are mostly tubular or dome-shaped. The larger tubes are made of ferromanganese micro-nodules, mineral grains, radiolarian fragments and sponge spicules, and are identified tentatively as the remains of agglutinating *Saccorhiza*. Some of the smaller tubes and domes also seem to be agglutinated particulates. The smallest structures I have observed are hollow opaque tubes and domes, rich in Fe and Mn (Fig. 1a). Scanning electron microscopy suggests that these structures are precipitated *in situ*; they are dark reddish brown in reflected light, compared with dark brown or black for other spheroids on the surface of nodules. There is evidence of other microscopic life forms on nodules (Fig. 1b). In some regions of the nodules, particularly the bottom surfaces, thin, fragile, transparent membranes form large (1–100 mm²) canopies under which there is no sedimentary debris. (Other evidence of life forms will be detailed elsewhere.)

Some *Saccorhiza* tubes had formed on all the nodules I examined. The tubes are often broken open and the remaining fragments partially filled or covered by ferromanganese hydroxyoxides (Fig. 1c). Some of the infilling

material seems to have formed *in situ*, and often has a distinguishable tube and dome structure (Fig. 1a). These observations suggest that the remains of the organisms inhabiting the nodules are incorporated into them as they grow, and that Mn micronodules are agglutinated to become part of the nodule.

If the structures produced by the organisms are preserved within the nodule, they would contribute to the overall growth of the nodule. The extreme of this hypothesis would be to consider nodules as a superstructure of surface-deposited tubes and fragments, within which would accumulate micronodules and matrix deposited organically or inorganically. To test this hypothesis, thin unsupported cross-sections of nodules were prepared. Opaque metal hydroxyoxides were leached away, leaving a fragile, nearly white framework of the original section (details to be presented elsewhere). Examination of this skeleton confirmed that preserved tubes are abundant inside nodules (Fig. 2). Preserved sections of complete tubes of *Saccorhiza* have been found, and assemblages of mineral grains of the appropriate size and arrangement are frequent. Fewks⁴ and Sorem⁵ have described features that are probably cross-sections of *Saccorhiza* tubes.

The preserved tubes seem to alternate with the concentric rings seen in Fig. 2, indicating that the onion-like structure of the nodules is a consequence of the particular type of remnant structure present. Alternatively, the zoning may be a result of diagenetic alteration of the nodule, perhaps under the influence of the microenvironment which is controlled by the presence of the tube material.

No leaching agent has been found that does not remove the Fe and Mn rich tubes and domes, and so direct observation of these structures has been impossible. It seems that they are either not preserved within the nodule (being altered into general matrix material) or become filled with a substance so chemically similar to themselves that leachants able to remove the filling also dissolve the structure.

The fragile remnant structure includes more than tubes and domes: there are globules of clay, thin stratiform sheets and many rather large (~ 0.1 mm) mineral grains. (Adjacent stratiform sheets can be confused with longitudinal cross-sections of tubular structures.)

The localisation of tubes and domes on parts of the

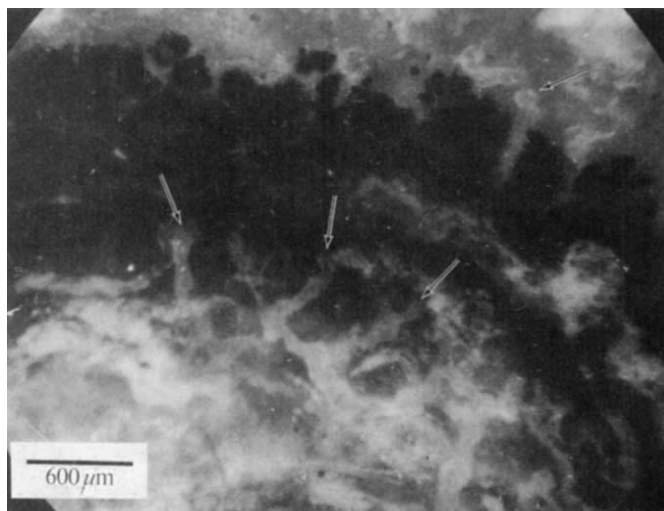


FIG. 2 Photomicrograph of tubes preserved in interior of a nodule and exposed by leaching. Leached material is nearly white, with no contrast so that dark-field photography was necessary, accounting for the poor contrast. The exposed tubes should be compared with those of Fig. 1d (arrow). Note the relationships of tubes and incompletely leached dark concentric rings shown in this cross-section.

nodule surfaces suggests that the organisms were 'active' at the time of collection. Most areas, however, yield no unfilled structures and are assumed to be 'dead' with respect to the relevant organisms. The greatest abundance (up to complete surface coverage) of 'active' structures occurs in regions of nodules believed to be near the sediment-water interface, suggesting that this type of growth could be responsible for the equatorial bulges in some nodules. Although all nodules investigated have had 'active' structures, 'dead' nodules with no discernible external biological produced surface structures, yet containing preserved tubes, might be expected.

It is unclear how the preserved structures were filled. Ehrlich *et al.* (ref. 6 and references therein) have adduced evidence that bacteria could play a role, but it is often suggested that slow, continuous inorganic precipitation of Mn and Fe is widespread on the ocean floor, leaving open the possibility that the infilling is entirely inorganic. The similarity of concentration ratios of several elements in adjacent sediments and nodules⁷ indicates that the deposition of the metal-rich phases in both nodules and sediments is similar, whatever the process.

Graham⁸ suggested that Mn nodules were of biological origin when he detected organic material in Mn crust from the Blake Plateau. He and Cooper⁹ also suggested a biological origin for the 'manganese-rich' deposits of the seafloor, citing their observation of Mn coating on samples of arenaceous foraminifer skeletons from the Atlantic Ocean. The suggestions have remained unproven, principally because Graham and Cooper were unable to rule out contamination with organic matter or inorganic precipitation of Mn. Glasby and Hodgson¹⁰ concluded from the low concentrations of organic pigments that organic influences were minimal in the formation of Mn deposits taken from the Indian Ocean. There is, however, no reason why structures such as I have found should contain significant concentrations of such pigments.

The abundance of biological structures throughout each nodule strongly suggests that the growth of deep-sea Mn nodules is aided by the activity of a diverse community of microorganisms. In an otherwise mushy environment, they build a solid superstructure within which ferromanganese oxides can accumulate.

This scheme fits well with reported observations. For example, if nodule development were dependent on biological activity, then, in the light of experience with other marine communities, nodules would be expected to be irregularly distributed geographically, which they are. They would further be expected to exhibit regularity in size, spacing and morphology within any one patch, which they do.

Radiometric studies show that nodules are older than the sediment on which they lie, and this might be due to the activity of the microorganisms. The ability of small organisms to move large objects is well documented by Darwin's description of earthworms moving stones. The presence of microorganisms on nodule surfaces reinforces suggestions that Mn nodules could be moved by predators searching for food².

It is often suggested that the internal structure of nodules is the result of alternating periods of growth and decay ('alive' and 'dead'). This would explain results obtained with Be¹⁰ (ref. 11 and references therein), which show that nodules only grow 10% of the time. Even when 'alive', the nodules do not grow over all their surface simultaneously. Because most of the organisms involved are very small, it would require a long time to produce a new surface over the entire nodule. These observations conform with growth rates determined by several workers (1–100 mm per 10⁶ yr).

Finally, if the communities of microorganisms vary from one locality to another, the nodules in the different areas might be expected to differ in shape, structure and composition. Again, such differences are common.

Of course I do not wish to imply that all constituents of Mn nodules are of biological origin. They may, in this and other respects, be analogous to coral reefs. But any model intended to explain the origin and development of deep-sea Mn nodules must take account of the extensive biological activity on their surfaces.

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J. GREENSLATE

*Scripps Institution of Oceanography,
Box 1529
La Jolla, California 92037*

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Photoperiodic responses in experimental hybrids of cocklebur

HYBRID intermediacy in night requirements for flowering has been reported in a study¹ of latitudinal variation of photoperiodic adaptation in cockleburs (*Xanthium strumarium* L.). The report stated that the data showed that different strains produced F1 hybrids photoperiodically intermediate between the parents and that segregation for critical night lengths was observed in the F2 generation. Ray (personal communication) feels, however, that the hybrid evaluation was possibly affected by facultative apomixis. I have also demonstrated^{2,3} intermediacy of the photoperiodic response in F1 hybrids in *Xanthium* but have not found evidence of apomixis in emasculated plants.

To determine the potential role of hybridisation in their wide distribution, cockleburs from different parts of the world were crossed after they were induced to flower under experimentally controlled conditions. Plants with diverse night requirements between 7.5 h and 10.75–11 h (refs 1, 4, 5) were hybridised with day neutral plants^{6,7} as well as with other photoperiodic types. The ease of producing F1 hybrids^{3,8,9} within and between the members of the eight morphological complexes of Löve and Dansereau¹⁰ extends to the production of F2 progenies; however, in crosses between the indigenous Eurasian complex, *strumarium*, and various American complexes, the F1 plants are only partially fertile^{11,12}.

TABLE 1 Photoperiodic adaptation of F1 hybrids involving the introduced *italicum* complex of Tahiti*

Selfed parental progenies (and morphological complex) and F1 hybrids†	Manifest night (h)‡	Apparent critical night (h)‡	Time of bud appearance outdoors in Texas §
Tahiti-A (<i>italicum</i>)	10.75–11.00	10.5–10.75	September 5–12
Tahiti-B (<i>italicum</i>)	11.00	10.75–11.00	August 25–September 8
F1 Tahiti × Pullman, Washington	9.25	9.00	June 28
Pullman (<i>oviforme</i>)	8.25	8.00	June 17–24
F1 Eureka, California × Tahiti	9.00	8.75	July 29–August 2
Eureka (<i>pennsylvanicum</i>)	8.25	8.00	July 4–8
F1 Tahiti × San Diego, California	10.00	9.75	July 29
San Diego (<i>italicum</i>)	10.00	9.50–9.75	July 22–29
F1 Tahiti × Wellington, Australia	10.25	10.00	August 8–19
Wellington (<i>italicum</i>)	10.5	10.0–10.25	August 19–26
F1 Tahiti × Bangalore, India	10.75	10.50	August 19–23
Bangalore (<i>chinense</i>)	11.00	10.50–10.75	August 26–29
F1 Tahiti × Bombay, India	10.75	10.50	August 23–26
Bombay (<i>chinense</i>)	11.00	10.75–11.00	September 5–12

* Tahiti burrs were originally collected near Maraa caves. The two selfed progenies were germinated from burrs produced in growth chambers¹⁴. The Tahiti plants show approximately 72% acetocarmine-stained pollen similar to that of some plants (65%) along the Tamaulipas coast of Mexico. All of the F1 hybrids involving a Tahiti parent had stained pollen within the range from 54 to 68%, most exceeding 60%. For the other parents, stained pollen exceeded 99% except for San Diego plants, 65%.

† In each cross except with Eureka, California the Tahiti plant is the pistillate parent.

‡ The manifest night under varying nights in the growth chambers is the dark period under which macroscopic floral buds become visible. The apparent critical night is a preceding dark period under which floral induction probably occurred. This terminology follows that of Ray and Alexander¹.

§ The time of bud appearance is for five plants of each selfed parental or hybrid progeny under outdoor garden conditions in Austin, Texas. The plants were germinated under continuous light and transplanted to natural daylengths in mid-June approximately six weeks after sowing of the burrs. The night length, which reaches a minimum near 9 h in mid-June, should have resulted in rapid floral induction of plants with night requirements less than 9 h. The Eureka, California plants and the Eureka–Tahiti hybrids were delayed in flowering possibly because of a response to high outdoor temperatures. Other California and Australia *pennsylvanicum* plants showed a delay in the time of expected bud production.

Selfed parental progenies were compared with hybrid progenies in growth chambers under fixed and/or varying dark periods with a day temperature of 30° C and a night temperature of 24° C. Although fixed photoperiods are necessary for establishing a critical night requirement, the increasing nights enable simultaneous testing of diverse responses. In most cockleburrs, the longer night accelerates bud development and, therefore, the manifest night¹ under which macroscopic floral buds appear is preceded by a shorter critical night for floral induction.

Five plants in each progeny were compared under each of two varying nights: 7.5 to 9.0 h, 8.0 to 10.0 h, 9.0 to 10.5 h, or 10.25 to 11 h. By determining the number of nights required for bud production in dark periods near the

inductive one, the apparent critical night¹ was estimated. In F1 studies, the dark period was increased 15 min at 5–10 d intervals, but for testing of F2 progenies the varying nights were increased 5 min every 5 d. The various F1 progenies were germinated and kept under continuous light for 6 to 9 weeks before the varying night comparisons. The Tahiti plants were 12 weeks from sowing of the burrs when initially exposed to 10.75 h nights. Selfed progenies of day neutral parents were germinated under fixed 16 h dark periods as well as under continuous light, and subsequently flowered and produced mature achenes in both conditions. Five to ten plants of each progeny were studied outdoors from mid-June in Texas.

F1 hybrids involving a day neutral parent and/or a parent

TABLE 2 Photoperiodic adaptation in hybrid progenies of *Xanthium* under varying nights*

Selfed parental progeny (and morphological complex) and hybrid progeny†	Number of plants with macroscopic floral buds in each varying night (h min)‡							Total number of plants
	935	940	945	950	955	1000	1100	
P1 Marfa, Texas (<i>italicum</i>)		1	3					4
P2 Lexington, Kentucky (<i>chinense</i>)				6	3			9
F1			1	4				5
F2(P1 × P2)(P1 × P2)	1	3	10	18	10	2		44
F2(P2 × P1)(P2 × P1)	1	3	7	24	11	1	1	48
ΣF2	2	6	17	42	21	3	1	92
P1 Wilberforce, NSW, Australia (<i>cavanillesii</i>)			6					6
P2 Manilla, NSW, Australia (<i>italicum</i>)							12	12
F2(P1 × P2)(P1 × P2)				13	9	3	1	26
F2(P2 × P1)(P2 × P1)				9	12	7	1	29
ΣF2				22	21	10	2	55

* Plants were 25 d from sowing of the burrs under continuous light when they were placed in a growth chamber nights of 9 h 20 min. The night length was increased by 5 min, (± 10s) each 5 d until it reached 10 h. The night length was subsequently fixed at 11 h to accelerate floral bud development. Test plants (25 d) produced macroscopic floral buds after 10 cycles of 16 h darkness. Experimental plants were 35 d from sowing at the first cycle of 9.5 h, the night expected to induce Texas *italicum* plants and Australia *cavanillesii* plants.

† F1 plants of the Australian cross were studied previously² and showed near midparent intermediacy.

‡ The critical night for floral induction probably preceded the manifest night by approximately 10 d, the Texas plants under nights of 9 h 30 min to 9 h 35 min and the Kentucky plants under 9 h 40 min to 9 h 45 min. In previous studies Marfa, Texas, plants² had indicated a critical night near 9.5 h and the Lexington, Kentucky plants¹⁵ near 9.75 h. In other studies the Wilberforce plants¹⁶ had shown induction under 9.5 h nights and the Manilla plants¹⁶ under 10 h nights. My present study suggests a critical night for Wilberforce near 9 h 35 min and for Manilla near 9 h 55 min.

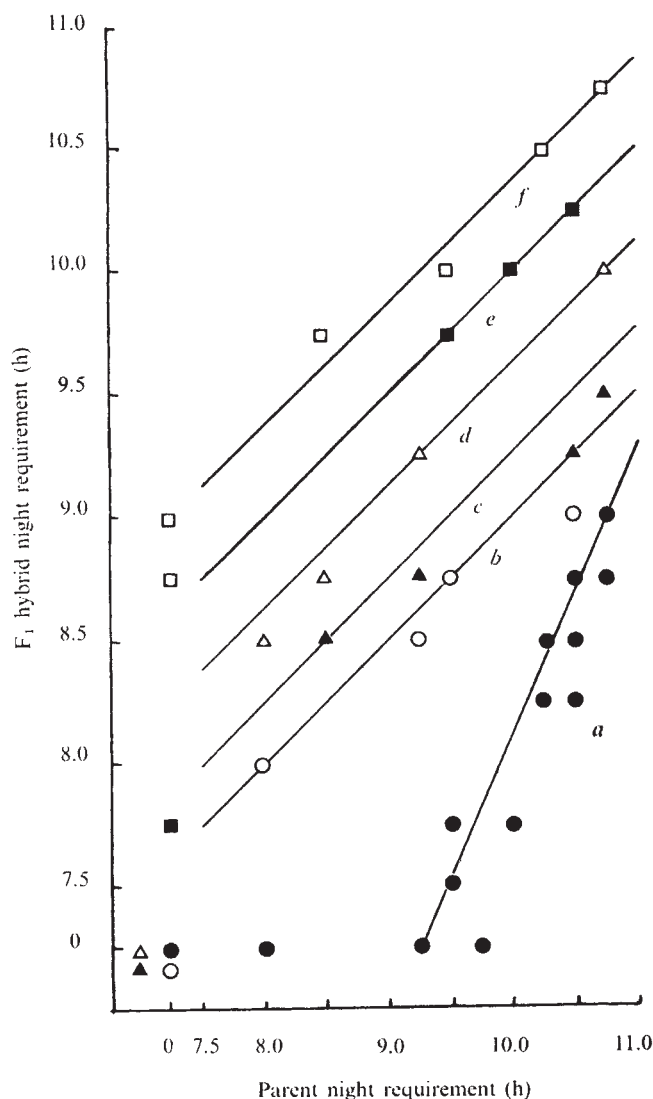


FIG. 1 Comparison of F1 hybrid night requirements for flowering. *a*, Hybrid responses with a day neutral parent (Varanasi, U. P.; Bangalore, Mysore, India) of the *strumarium* morphological complex (●). Other hybrid responses are with one of the following parents: Washington, *oviforme* (○); Italy, *italicum* (▲); Australia, *pennsylvanicum* (△); Australia, *italicum* (■); and Mexico, *italicum* or Bombay, India, *chinense* (□). The responses of hybrids between parents with night requirements are plotted along lines representing midparental intermediacy; *b*, × 8.0 h; *c*, × 8.5 h; *d*, × 9.25 h; *e*, × 10.0 h; *f*, × 10.75 h. Other parental plants used in the experimental crosses include: Eureka, California, *pennsylvanicum*, 8.0 h; Australia, *cavanillesii*, 9.5 h; Kentucky, *chinense*, 9.75 h; San Diego, California, *italicum*, 9.5–9.75 h; Brazil, *cavanillesii*, 10.25 h; Australia, *chinense*, 10.5 h; India, *chinense*, 10.25–10.5 h. Points on the graph represent the shorter night if two are indicated for a parental response.

with a critical night of 10.75–11 h show a wide range of photoperiodic adaptation (Fig. 1). The hybrids with a day neutral parent have an adaptive range from day neutrality to a 8.75–9 h night requirement¹³. Those involving a parent with a night requirement of 10.75–11 h show adaptation to nights of 8.75–9 h or longer. The crosses between day neutral plants of India and plants of Mexico¹⁴ with the 10.75–11 h requirement produced the hybrid response to 8.75–9 h.

Approximate midparent intermediacy of the F1 hybrid response was demonstrated by most crosses involving two parents with a night requirement. Hybridisations included in Fig. 1 are those involving the *oviforme* complex from Wash-

ington, 8.0 h; the *italicum* complex from northern Italy, 8.5 h; the *pennsylvanicum* complex from Australia, 9.25–9.5 h; the *italicum* complex from Australia, 10.0 h; and either a Mexican plant in the *italicum* complex or an Indian plant in the introduced *chinense* complex, 10.75–11 h. Midparent intermediacy was also demonstrated by a series of crosses³ involving plants from America and introductions to Australia with adaptation to various night lengths from 9.25 to 10.5 h.

F1 hybrids involving a Tahiti parent showed responses not reflecting midparent intermediacy (Table 1). Hybrid night requirements were slightly shorter than those of either parent in crosses with Bangalore and Bombay plants. The response of hybrids with San Diego and Wellington parents were similar. The hybrids with parents requiring 8 h nights showed near midparent intermediacy but were closer to the parent with the shorter night requirement. The hybrid responses may indicate a temperature interaction, differences in ripeness-to-flower (maturity) responses and/or partial dominance by one parent. Hybrids involving Hong Kong *strumarium* plants with a 9.25–9.5 h night requirement also failed to show midparent intermediacy^{12,13}. With an American parent requiring a shorter night, the hybrid response was to a similarly short night. Hybrids involving an American parent with a longer requirement, 10.25–10.5 h, were intermediate in response but more similar to that parent.

F2 progenies examined under varying nights showed segregation of the photoperiodic response (Table 2). In the cross involving *italicum* from Marfa in western Texas² and *chinense* from Lexington, Kentucky¹⁵ the parental requirements were separated by less than 15 min and the F2 generation, consisting of 92 plants, responded to nights differing by approximately 30 min. The cross between *italicum* and *cavanillesii* introductions^{3,15} to Australia included an F2 progeny of 55 plants and a range of response to nights differing by approximately 20 min. Burr morphology and sesquiterpene lactone chemistry also show segregation in the F2 generation but the studies suggest no genetic linkage of either characteristic with photoperiodic response (my unpublished results). The F3 generation and other F2 progenies are being investigated for additional information about photoperiodic adaptation.

The F2 progenies of day neutral plants in the *strumarium* complex and *chinense* plants introduced to India^{7,11} and to Australia^{15,16} consisted of few plants because of partial fertility of the F1 hybrids^{11,12}. Tested under natural day-lengths (and dark periods lengthening from 9 h in mid-June) in an outdoor garden following 6 weeks under continuous light in growth chambers, none of the F2 plants showed day neutrality. Each of the 13 plants that germinated from a total of 100 burrs produced on five F1 plants, showed a flowering reaction suggesting adaptation to nights shorter than 9.75 h. The selfed progeny of the day neutral parent flowered in growth chambers under continuous light; the selfed progeny of the *chinense* parents with a 10.5 h night requirement produced macroscopic floral buds in late August; the F1 and eight of the F2 plants in early July and the remaining five F2 plants before the end of July. The F1 had shown a requirement of nights of 8.25–8.75 h previously¹³.

The intermediate photoperiodic response in F1 hybrids and the segregation for critical night length in the F2 generation suggest quantitative inheritance of the dark requirement for flowering. The hybridisation results also suggest that day neutrality in *Xanthium* is not dominant over a photoperiodic response as was indicated by the original studies of inheritance of photoperiodism in *Nicotiana*¹⁷. Day neutrality in *Xanthium* seems to result from greater negative physiological effects. Conversely, an imbalance towards increasing positive effects seems to produce the broad range of increasing night length requirements.

The diversity of photoperiodic adaptation in wild populations of *Xanthium* and the range of hybrid response may allow a broad base for future study of photoperiodic mecha-

nisms. Current physiological studies in *Xanthium* have concentrated almost exclusively on one or more strains from the vicinity of Chicago, Illinois¹⁸ with a critical dark period usually given as 8.3–8.5 h. The actual mechanisms of photoperiodic control has been elusive but many physiological aspects of the dark induction have been reported for the Chicago strain(s)¹⁹. The present hybridisation studies were not intended to investigate the photoperiodic mechanism, but the results suggest that the photoperiodic response in *Xanthium* may be the total of 'negative' and 'positive' enzymatic effects and may also involve a timed reaction.

It seems unlikely that photoperiodic adaptation in *Xanthium* extends beyond the range of 7.5 to 11 h. Day neutrality is thinly separated from photoperiod controls at the short end of the range in indigenous European plants (my unpublished results) and tropical plants with a requirement near 11 h seem to have secondary controls involving temperature and maturity effects¹⁴. The results of hybridisation are providing insight into the adaptive capacity of *Xanthium* as a worldwide coloniser of habitats disturbed by man. In India the diversity of photoperiodic responses^{6,7} can be explained as the result of hybridisation between day neutral indigenous plants and plants with photoperiodic responses introduced from America^{11–13}.

I thank Drs A. Lang, D. Löve, P. M. Ray, F. B. Salisbury, G. L. Stebbins, and J. A. D. Zeevaart for their advice. Dr Löve has examined burrs of most of the parental plants and many of the hybrids and has agreed with the treatment of the morphological complexes.

C. McMILLAN

Department of Botany,
and Plant Ecology Research Laboratory,
University of Texas at Austin,
Austin, Texas 78712

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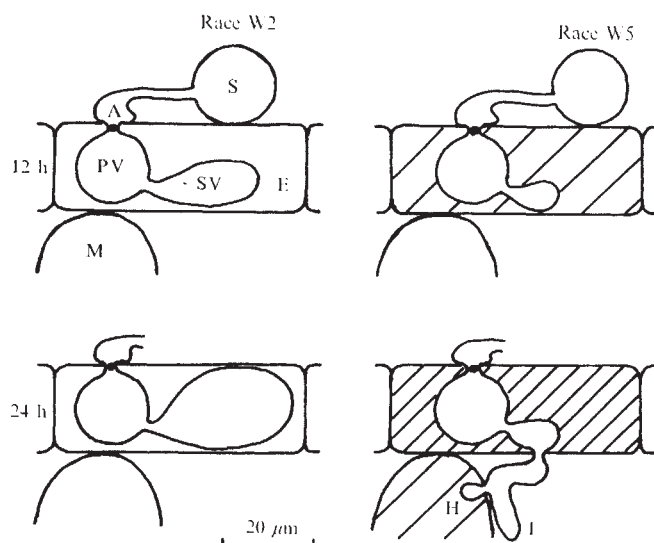


FIG. 1 Diagrammatic representation of the development of a compatible race (W2) and an incompatible race (W5) of *Bremia lactucae* in the lettuce cultivar Avondefiance. Primary and secondary vesicles are drawn to scale, measurements being derived from the means of ten replicates. Shading represents the extent of granulation and melanisation of the host cytoplasm. S, Spore; A, appressorium; PV, primary vesicle; SV, secondary vesicle; I, intercellular hypha; H, haustorium; E, epidermal cell; M, mesophyll cell.

*et al.*³, who have suggested that the hypersensitive reaction of wheat to rust fungi is not the cause, but a consequence, of the earlier inhibition or death of the invading fungus. Király *et al.*⁴, on the basis of indirect evidence, have extended this suggestion to include the resistance of bean to *Uromyces phaseoli* (rust) and potato to *Phytophthora infestans* (blight), and have questioned whether the term 'hypersensitive reaction' of hosts in connection with plant resistance has been correctly used in the past. Since this is a continuing debate among plant pathologists, we consider

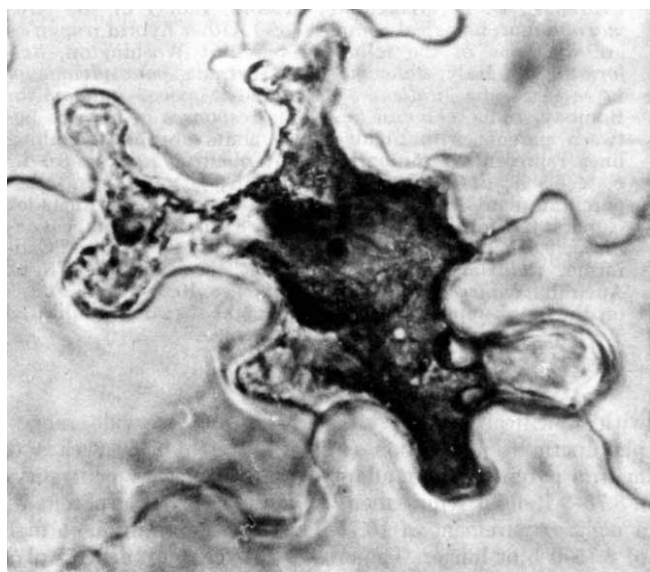


FIG. 2 Surface view of the hypersensitive reaction of an epidermal cell of the lettuce cultivar Avondefiance 60 h after inoculation with the incompatible race W5 of *Bremia lactucae*. Cells adjacent to the infected cell are apparently unaffected.

Hypersensitivity as the primary event in resistance to fungal parasites

THE hypersensitive reaction of plants to infection by incompatible strains of biotrophic fungi (organisms which, as parasites, derive their nutrients from living tissues¹) involves the rapid necrosis of one or more cells at the penetration site, accompanied by restriction of the invading fungus. It is generally believed that this reaction by the host leads to a disruption of nutrient supplies to the fungus and the production of fungitoxic metabolites (for example, phytoalexins), resulting in the cessation of hyphal growth². This traditional view has, however, been challenged by Brown

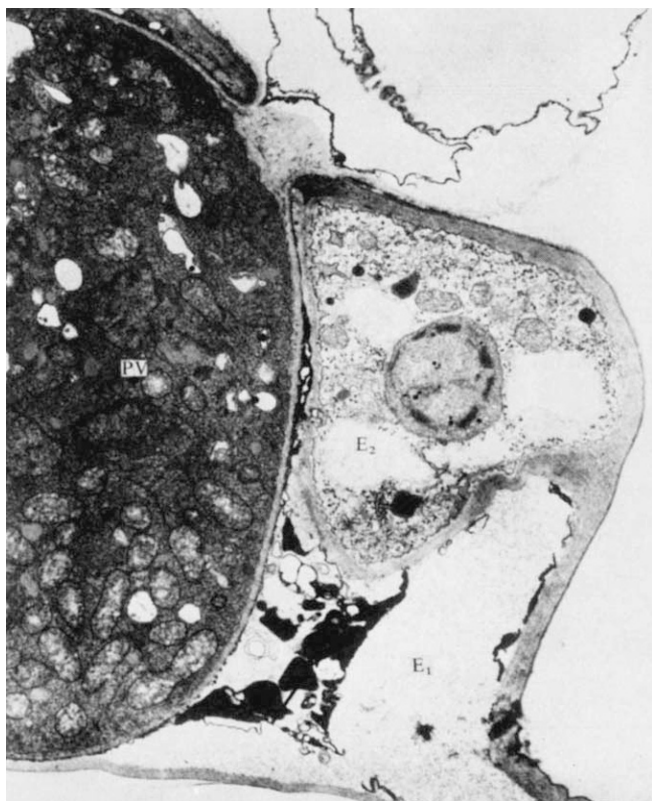


FIG. 3 Electron micrograph showing a primary vesicle (PV) of an incompatible race of *Bremia lactucae* (race W5) in an epidermal cell (E_1) of the lettuce cultivar Avondefiance. Note the complete disruption of the membranes and cytoplasm of this cell (arrowed) and the apparent normality of the cytoplasm of the fungus and the adjacent, non-penetrated cell (E_2).

it pertinent to report here information which accords with the traditional view of the significance of the hypersensitive reaction. We show that in the resistance of lettuce (*Lactuca sativa* L.) to incompatible races of the downy mildew fungus *Bremia lactucae* Regel, death of penetrated host cells is the primary event, preceding the cessation of fungal growth by several hours.

During a study of the interaction between four races of *B. lactucae* and five cultivars of lettuce we observed that incompatibility was normally accompanied by a hypersensitive reaction in the host. The time course of the infection process was followed in detail after inoculating the abaxial surfaces of cotyledons of the lettuce cultivar Avondefiance with aqueous suspensions of conidia of *B. lactucae* race W2 (compatible) or W5 (incompatible). Light microscopy showed that the germ tubes of both races penetrated epidermal cells and by 12 h after inoculation had produced a spherical primary vesicle and an elongated secondary vesicle⁵. The primary vesicles of both races developed at the same rate. Growth of the secondary vesicle of the incompatible race, however, was markedly retarded compared with that of the compatible race (Fig. 1); the former was fully expanded ($5 \times 9 \mu\text{m}$) by 12 h and the latter continued to enlarge for 24 h, reaching a final size of $14 \times 26 \mu\text{m}$. Observations at intervals up to 60 h showed that the compatible race caused no necrosis of the epidermal cells it penetrated. In contrast, the epidermal cells entered by the incompatible race showed progressive granulation and melanisation, although adjacent non-penetrated cells remained apparently unaffected (Fig. 2). From 24 h onwards, the compatible race formed an intercellular hypha giving rise to an extensive

mycelium bearing haustoria. The incompatible race, however, initiated an intercellular hypha as early as 12 h, although this stopped growing by 24 h, after producing approximately three haustoria which caused a hypersensitive reaction in the cells they entered.

Cytological examination of both strains of the fungus from spore germination onwards showed that for the first 12 h there was no significant increase in the number of nuclei per colony. Thereafter, the number of nuclei increased in colonies of the compatible but not of the incompatible race. Nuclear division in *B. lactucae* is probably dependent on the establishment of a biotrophic relationship between host and fungus and the failure of the nuclei to divide in the incompatible race is a reflection of its inability to establish such a relationship.

Electron microscopy of infected cotyledons showed that in compatible interactions the host cell plasmalemma was invaginated to accommodate both the primary and secondary vesicles of the fungus, and there was no evidence of cytoplasmic damage⁵. In contrast, within 4 h the membranes and cytoplasm of epidermal cells penetrated by the incompatible race were severely disrupted (Fig. 3), although adjacent cells were apparently normal. The fungal cytoplasm also appeared perfectly normal at this time, being dense with apparently functional organelles.

These observations make it clear that the primary event in the interaction between Avondefiance and the incompatible race W5 of *B. lactucae* is the rapid death of the penetrated cells. The fungus continues to grow for at least 12 h after this, although it too eventually dies. The most likely cause of death of penetrated cells is a rapidly-acting toxin produced by the healthy fungus during the early stages of the interaction. Since adjacent cells are not killed, this toxin could be a molecule which is closely associated with the fungal cell wall, or which cannot diffuse in sufficient quantity through host cell walls.

In the specific example reported here, light and electron microscopic studies during the very early stages of the host-parasite interaction have produced a clear sequence of events during the hypersensitive reaction to infection by an incompatible fungus. It is likely that more than one mechanism for hypersensitive resistance in plants exists, but it would be interesting to know whether the contradictory conclusions of earlier studies^{3,4} would be supported by microscopic observations during the early stages of the host-parasite interactions.

Experimental details of this report will be published elsewhere. We thank Professor P. W. Brian, for discussions.

D. J. MACLEAN
J. A. SARGENT
I. C. TOMMERUP
D. S. INGRAM

ARC Unit of Developmental Botany,
181A Huntingdon Road,
Cambridge CB3 0DY

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New species of *Dryopithecus* from Kenya

Two new species of Pongidae, assigned to a new subgenus of *Dryopithecus*, are here described for the first time. These assignments are based mainly on new specimens discovered recently in Kenya, but some other specimens^{1,2} are reassigned to the hypodigms of the new species. A more complete revision of the African early Miocene dryopithecines is in preparation.

Three species of *Dryopithecus* from East Africa—*D. africanus*, *D. nyanzae*, and *D. major*—have been accepted as valid species, and are grouped in the subgenus *Proconsul* (Hopwood)³. The two new species are grouped in another subgenus to distinguish them from the other African and Eurasian species of *Dryopithecus*. Moreover, *Limnopithecus legetet*, which has pongid affinities, both dentally and post-cranially, and which differs mainly in size from the species of *Dryopithecus*, is also included in the Dryopithecinae. As the subgeneric affinities of this species are not clear it seems best at this stage to retain it as a separate genus of the Dryopithecinae. The other species assigned to *Limnopithecus*, '*L. macinnesi*', is retained in the Hylobatidae¹, so that it will be necessary to change the generic classification of this species. Thus there are two genera and six species of the Pongidae, Dryopithecinae, and one genus and species of the Hylobatidae, now recognised in the early Miocene of East Africa. The dentitions of the six dryopithecine species range in size from slightly smaller than those of the gibbon to about the size of those of a female gorilla.

Le Gros Clark and Leakey¹, when describing a maxilla fragment assigned to *Proconsul africanus* (field no. 415/47, KNM-SO 401), noted its aberrant features and stated that further similar material might make a new species necessary. Such new material came in Leakey's 1966 collection from Songhor in the form of a complete palate which I described in 1970 (ref. 2). At that time, as my study of the total sample was incomplete, I referred it to *Proconsul* sp. with the intimation that it probably belonged to a new species; this view is now found to be justified. The entire dentition, based on a total of 79 specimens, is known and a complete half mandible has just been found at Songhor (KNM-SO 1112) so that the lower dentition is now as well known as the upper. This new specimen is here described as a new species, *Dryopithecus gordonii*.

Another recent discovery, this time from Rusinga Island, was a partial maxilla and upper dentition (KNM-RU 2058)

TABLE 1 Measurements of M¹ of *D. gordonii* and *D. vancouveringi*

	md	bl	bl md
<i>D. gordonii</i>			
MW 52	8.4	8.0	95.3
SO 401	8.1	7.9	97.5
487	8.4	7.9	94.1
700	8.5	8.1	95.3
930	8.1	8.5	104.9
931	8.1	8.0	98.8
932	7.8	8.2	102.6
Mean	8.2	8.1	98.0
Number	7.0	7.0	7.0
sd	0.24	0.21	4.05
<i>D. vancouveringi</i>			
RU 1778	7.0	6.8	97.2
1801	6.4	6.6	103.0
2058	6.8	6.5	95.6
SO 944	6.8	6.4	94.2
1134	6.5	6.5	100.0
MB 125	7.1	6.7	94.4
Mean	6.8	6.6	97.0
Number	6.0	6.0	6.0
sd	0.27	0.15	2.48
Value of <i>t</i>	9.9	13.8	
Degrees of freedom	11.0	11.0	
Probability (<i>P</i>)	<0.001	<0.001	

which, although almost identical morphologically to *D. gordonii*, is much smaller. Unfortunately the smaller form is known only from the upper dentition and from only seven specimens. One of these, a maxilla fragment (field no. R 593/47, KNM-RU 1778), had previously been allocated¹ to *L. macinnesi*. There are no morphological differences between these small specimens and *D. gordonii*, but examination of the ranges of variation and significance tests established that samples are sufficiently different in size to make it unlikely that they come from one population ($P < 0.001$). The smaller sample is therefore described here as a new species, *Dryopithecus vancouveringi*.

The similarity of the two new species is illustrated in Fig. 1 where the upper tooth rows have been reduced to the same P4-M3 length. For comparison the equivalent tooth rows of *D. africanus* and '*L. macinnesi*', which correspond most closely in size to *D. gordonii* and *D. vancouveringi* respectively, are

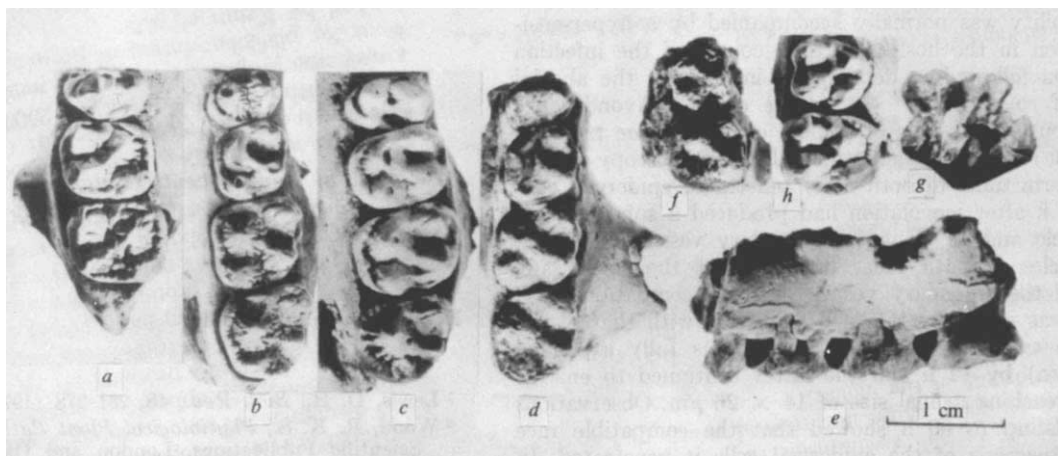


FIG. 1 Occlusal views of upper dentitions. a, *D. africanus*, P4-M2, KNM-RU 2036; b, *D. gordonii*, P4-M3, KNM-SO 700; c, '*L. macinnesi*', P4-M3, KNM-RU 1849; d, *D. vancouveringi*, P4-M3, KNM-RU, 2058; e, *D. vancouveringi*, P4-M3 (lateral view), KNM-RU 2058; f, *D. vancouveringi*, dp4-M1, KNM-RU 1778; g, *vancouveringi*, dp4-M1 (lateral view), KNM-RU 1778; h, *D. major*, dp4-M1, KNM-SO 542. d, e, f, and g are all to the same scale as indicated, but the other specimens are reduced or enlarged to the same P4-M3 length as in d, or to the same dp4-M1 length as in f.

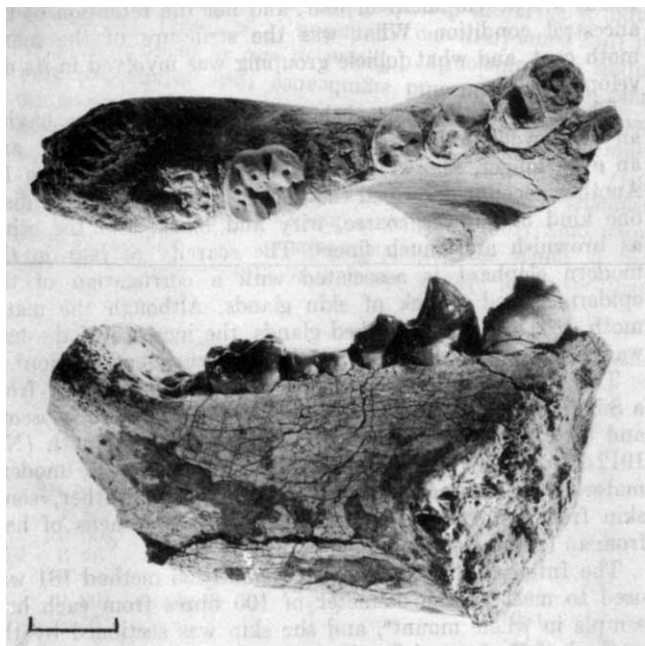


FIG. 2 Occlusal and lingual views of the new mandible of *D. gordonii* (KNM-SO 1112).

also included. The figure illustrates the distinctive morphology of the two new species, particularly the molariform premolars, with large lingual and distal cingula, the elongated upper molars (also shown in the tooth dimensions given in Table 1), the large size of the protocone on the upper molars, and the increase in molar size from M1 to M3. Other features not immediately evident from the figure are the lowness of the molar and premolar cusps, the degree of wrinkling of the occlusal surfaces, and the relatively marked wear gradient on the molars. Other features which are present in the larger sample of *D. gordonii* but which are not known for *D. vancouveringi* include the remarkably high-crowned structure of the incisors and to a lesser extent the canines, the elongated and wrinkled lower molars, and the large size and depth of the mandible (Fig. 2). In all these shared features, the two species differ from all other species of *Dryopithecus*, so they are here combined into a single higher taxonomic category. Since recent usage in pongid taxonomy has been to recognise subgeneric rather than superspecific groups³, this category was selected, and the name *Rangwapithecus* was chosen. When more is known about the Eurasian dryopithecines, and in particular when better upper dentitions and skulls are found, it may well be necessary to resurrect some of the genera sunk by Simons and Pilbeam³, and this may include establishing *Rangwapithecus* as a valid genus.

Diagnosis of the two new species are as follows:

Subgenus: *Rangwapithecus* subgen. nov.

Diagnosis: species approximating in size to the gibbon and siamang. Incisors high crowned and relatively very narrow compared with (*Proconsul*) and (*Dryopithecus*). Upper molars and premolars elongated, the molars usually longer (mesiodistally) than broad, low cusped and the occlusal surface often has more secondary wrinkling than in (*Proconsul*). Upper molars increase in size from M1-M3 and premolars from P3-P4 unlike both (*Proconsul*) and (*Dryopithecus*). No reduction of M3. Lower molars and premolars also elongated. The molars have a marked wear gradient, such that M1 may have dentine exposed on the occlusal surface when M3 is only just coming into wear, unlike the condition in (*Proconsul*) and (*Dryopithecus*). Strong lingual cingula are developed on all the upper molars and premolars, and the premolars also have a prominent distal cingulum. Zygomatic process is set very low over M1/2. The floor of the maxillary sinus is greatly extended.

Contained species: (1) *D. gordonii* sp. nov. (2) *D. vancouveringi* sp. nov.

(1) *Dryopithecus* (*Rangwapithecus*) *gordonii* sp. nov.

Holotype: KNM-SO 700 maxilla with complete upper dentition except for the incisors. Housed at the Kenya National Museum, Nairobi.

Locality: Songhor, Kenya.

Reference: Andrews², not named but referred to *Proconsul* sp. (Individual specimens attributed to this taxon have been assigned in the literature to *Limnopithecus macinest*, *Proconsul africanus* and *Proconsul nyanzae* (ref. 1).)

Distribution: Early Miocene. Most common at the Tinderet site of Songhor but a few specimens are known from the Rangwa sites of Rusinga and Mfangano Islands.

Hypodigm: seventy-nine specimens which cover the entire dentition, the mandible except for the ascending ramus, the maxilla and palate. A few postcranial fragments may belong to this species. BM(NH) M 16637; KNM-RU 1686, 1692, 1722, 1768, 1833, 1894; KNM-MW 52; KNM-SO 89, 373-5, 377, 383, 401, 419, 420, 428, 434, 445, 449, 450, 463, 464, 467, 473, 475, 486-8, 507, 521-3, 531, 540, 550-3, 555-63, 575, 577, 578, 583, 588, 590-3, 700, 904-9, 921, 923-5, 930-2, 938, 943, 945, 948, 1081, 1112, 1225.

Diagnosis: a species of *Dryopithecus* intermediate in dental size between the siamang and pygmy chimpanzee; similar in size to *D. africanus*. Upper teeth similar to those of *D. vancouveringi* as described in the subgeneric diagnosis, differing only in larger size. In the lower dentition the incisors are very high crowned and narrow; the canine is high crowned and bilaterally compressed; the P3 is very bilaterally compressed with nearly parallel buccal and lingual sides; the P4 is elongated; the M1-M3 are also elongated, the cusps are low, the buccal cusps are divided by deep buccal sulci like the condition in the gorilla, the occlusal ridges and buccal cingulum are poorly defined, and secondary wrinkling is often present. Mandibular body and symphysis relatively deep and robust, prominent superior transverse torus, no inferior torus. P3-M3 lengths are, upper 40 mm and lower 44 mm for single specimens. Upper M1-M3 length is 29 mm. Named in honour of H. L. Gordon, formerly Government Medical Officer at Koru, who collected many of the early specimens at Koru.

(2) *Dryopithecus* (*Rangwapithecus*) *vancouveringi* sp. nov.

Holotype: KNM-RU 2058 left maxilla with crowns of P4-M3. Housed at the Kenya National Museum, Nairobi.

Locality: Rusinga Island, Kenya.

Distribution: Early to ?middle Miocene at the sites centred on Rangwa: Rusinga and Mfangano Islands; and the Tinderet site of Songhor. It also may occur at Maboko Island.

Hypodigm: seven specimens which cover only the upper postcanine dentition and parts of the maxilla. Also referred provisionally to this species are three specimens from Maboko Island, KNM-MB 53, 125, 148. KNM-RU 1778, 1801, 2058; KNM-MW 48; KNM-SO 942, 944, 1134.

Diagnosis: a small species of *Dryopithecus* approximately the dental size of the siamang. It is like *D. gordonii* in morphology, as defined in the subgeneric diagnosis, but differs from it in size. The M1 is significantly different from the M1 of *D. gordonii*. The upper tooth row lengths are M1-M3, 22 mm and P3-M3, estimated 31 mm for a single specimen. Named in honour of Judith and John Van Couvering who found the type specimen in 1968 at the Kaswanga Point deposits of Rusinga Island.

The six species of dryopithecines apparently represent a Miocene radiation of the Pongidae in Africa. There are several degrees of differentiation between the species. The species pairs *D. nyanzae/D. major* and *D. gordonii/D. vancouveringi* probably represent near contemporary speciation, inasmuch as the members of each pair are largely allopatric in distribution and differ from each other principally in size. *D. africanus* is much more distinct, both in size and morphology, from the *D. nyanzae/D. major* pair, and it is sympatric with *D. nyanzae*: it is probable that it represents an older stage in speciation. Finally, the distinctive morphological difference between the species assigned to the two subgenera and genera must represent a still more ancient divergence.

The Miocene proliferation of pongid species in Africa seems strange in the light of the distribution of present day African pongids. Rather, closer evolutionary parallel might be with the later Cainozoic radiation of African cercopithecine monkeys, which are even more diverse than were the Miocene pongids. The fossil pongids are known only from one corner of their range in Africa; it seems unlikely from geographical

and palaeogeographical evidence that East Africa represented the centre of the Miocene pongid radiation, and the main centres of distribution were more probably in West and Central Africa (as are those of modern monkeys). No Miocene pongids have been recovered in these regions, but it is quite possible that if they were, the pongid radiation would be seen to be at least as rich and diverse as is the cercopithecine radiation of today.

The relationship of these fossil hominoid primates with the living ones remains uncertain. In my opinion it is no longer feasible to suggest direct ancestral-descendant relationships between fossil and living species⁴. Presumably one or more of the Miocene species was ancestral to the later pongids, but which this was, and whether one or more species was also ancestral to the Eurasian dryopithecines, can not be known from the available evidence.

I thank Drs D. R. Pilbeam and A. C. Walker for help with the preparation of this note. I also thank the Wenner-Gren Foundation for Anthropological Research for financial support, the Boise Fund for support of field work and the Museum Trustees of Kenya for permission to work on this material.

PETER ANDREWS

National Museum,
PO Box 40658,
Nairobi, Kenya

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Hair of the mammoth

THERE have been few detailed studies of the frozen remains of woolly mammoths (*Elephas primigenius*)¹. Here I describe a study of evolutionary change based on the grouping of the hair follicles and the distribution of hair diameters. This approach has hitherto been possible only with domestic animals².

The ancestral elephant *E. meridionalis*, a warm latitude forest dweller of the Early Pleistocene, gave rise to two lines. One remained in the same environment and underwent only moderate evolutionary change, giving rise to the straight-tusked *E. antiquus* of the Middle and Late Pleistocene. The other line evolved more rapidly, passing through two species stages in the same time; *E. trogontherii*, a cool steppe dweller of the Middle Pleistocene, and *E. Primigenius*, the woolly mammoth of the Arctic tundra in the Late Pleistocene¹.

A notable adaptation of the mammoth to a cold climate was the development of a long, dense coat. The ancestor of the elephants must have had hair, but it may already have been as much reduced in *E. meridionalis* as it is in modern ele-

phants. The coat of the mammoth is therefore likely to represent a redevelopment of hair, and not the retention of the ancestral condition. What was the structure of the mammoth coat, and what follicle grouping was involved in its development?

The coat of the mammoth has been described as having an underwool, a longer, coarser yet fluffy outer hair, and an even longer, scarcer, bristly but flexible hair³ (Table 1). Another account described the underwool as soft and reddish, one kind of hair as coarse, wiry and black, and the other as brownish and much finer⁴. The scarcity of hair in the modern elephant is associated with a corrugation of the epidermis, and a lack of skin glands. Although the mammoth, too, apparently lacked glands, the increase of the hair was associated with a loss of the epidermal corrugation⁵.

The ancient material available comprised some hair from a Siberian mammoth about 10,000 yr old sent from Moscow, and three samples of skin from the Liakhov mammoth (No. 1912-23) in the Palaeontology museum, Paris. The modern material for comparison was some elephant leather, some skin from an African elephant, and two specimens of hair from an Indian elephant.

The International Standards Organisation method 181 was used to measure the diameter of 100 fibres from each hair sample in whole mount⁶, and the skin was sectioned by the method of Ryder and Stephenson⁶, after treatment in Sandison's softening fluid^{7,8}.

It has been concluded⁵ that no fading has occurred in the colour of mammoth hair. By electron spin resonance⁹ the Moscow sample had been shown to have a concentration of free radicals equal to that in white wool, and so it was concluded that it had been white, and that the yellow-brown colour was merely discoloration. It seems unlikely that the mammoth was in fact white, since stomach contents indicate that it was vegetarian like the musk ox¹, and only predators or prey in the Arctic turn white in winter.

Although I was unable to detect pigment granules, the coarser mammoth hairs were darker. A feature common in double-coated mammals, this suggests that the hairs were originally pigmented, the pigment having become diffuse with time. Chemical degradation has been demonstrated in mammoth hair¹⁰.

The hair diameter distribution of the ancient and modern samples is shown in Table 2. The Moscow sample had a double coat but owing to its irregular nature, the length of the underwool could not be measured. The outer hair was shorter than the shorter (20 cm) of the two lengths quoted⁴ although not much less than the longest (12 cm) in Neuvill's unknown specimen⁵ (Tables 1 and 2). The lack of the longest hairs (45 cm) cannot be due to breakage, because no hairs of commensurate coarseness were present in either the Moscow or Liakhov samples (Table 2). Their absence could be due to shedding some time before death, which could imply death at the end of winter, and not at the end of summer as previously surmised¹.

The three samples of flank skin were of similar thickness: fresh, 23 mm; leather, 22 mm; Liakhov, 22 mm (leg only 12 mm). It was impossible to section the leather. The fresh

TABLE 1 Previous measurements

Source	Specimen	Underwool length	Outer hair length	
			Short	Long
Lang ³		5 cm	45 cm	longer (diameter ≤ 1 mm)
Quakenbush ⁴		3.8 cm (red)	15-20 cm (brown)	45 cm (black and bristly)
Neuvill ⁵	unknown	2.5-3 cm (40-75 μ m diameter)	12 cm (90-325 μ m diameter) red or black	
	Liakhov (leg)	light yellow	25* cm light red (blond) at base	

* Hair length confirmed in present study (see Table 2).

TABLE 2 Coat measurements

Specimen	Length (cm)	Range	Diameter (μ m) mode	Mean $\pm$ s.d.	CV (%)	Distribution
Moscow	10	24-132	40	56.8 $\pm$ 24.7	43.5	skewed-to-fine
Liakhov (leg)—straight	25	26-110	40	50.0 $\pm$ 15.8	31.5	skewed-to-fine
Liakhov (flank)—curly	stubble only	40-114	60	66.5 $\pm$ 15.0	22.6	slightly skewed
Indian elephant (body)	13	114-586	224	293.8 $\pm$ 104.4	35.5	continuous*
Indian elephant (head)	15		$\sim$ 500			

* Although to naked eye the few coarser ones stand out among the remainder

skin confirmed the corrugation⁵ of the epidermis, which had closely-packed pillars about 1 mm high resembling the villi of the intestine but hexagonal in section. The pillars were grouped into clusters separated by channels, and the hairs emerged at the junction of several channels. The hair density was 2 to 3 cm⁻², and were of two sizes, suggesting origination from primary central (larger) and primary lateral (smaller) follicles. The presumed centrals were deeper than the presumed laterals, extending 4 to 5 mm into the skin.

Sections cut parallel to the skin surface revealed sparse, round, non-medullated hairs with no glands or erector muscles. The follicle density was 6 cm⁻² (that is, two groups). The trios had a V configuration (90° angle) with the larger central follicle at the base and the two laterals at the tips of the arms, which were 2 to 5 mm long. At least one central follicle had a surrounding sinus which suggests that it was a tylotrich (special sensory) follicle¹¹.

The best sections of the Liakhov skin came from the leg. The collagen fibres were very coarse, and stained green with picro-indigo carmine, as found previously with undecayed remains⁸. Follicle debris stained maroon with basic fuchsin. The hairs exhibited slight basophilia, staining orange rather than yellow, with picric acid and fuchsin.

The hairs in the leg skin were noticeably circular in section and of two widely differing sizes (outer coat and underwool). One fine group of 15 ranged from 48 to 76 μ m in diameter (mean 62 μ m), and a coarser group of five ranged from 88 to 180 μ m (mean 129 μ m). Both groups were thus coarser than the similar hairs measured immediately above the skin surface (Table 2).

There were a few follicle groups with trios of larger 'primary' follicles in which the laterals were 0.2 to 0.3 mm from the central. Between the central and the lateral were smaller 'secondaries' (Fig. 1). Distributed at random were a few even larger empty follicles, one of which had a collapsed lumen measuring 140 $\times$ 400 μ m. These could be the source of the much larger hairs recorded by previous workers.

The central hair of the 'primary' trio in Fig. 1 is 68 μ m, whereas the lateral is 128 μ m, in diameter. The 'secondaries' between are 50, 56 and 62 μ m in diameter. The hairs within the skin seemed to have narrowed preparatory to shedding, in spite of the observation that those above the surface were finer. For example, the hairs within the skin had only a tiny medulla, although there was little medullation in either the hair above the surface, or in that of the modern elephant. These hairs are remarkably coarse to lack a medulla. Neuville⁵ mentioned elliptical as well as circular hairs, and variable medullation, although his measurements show that any medulla occupied no more than one-third of the width of the hair.

The Liakhov flank had more open connective tissue, and this seemed to be the cause of a loss of hairs during sectioning. No groups were evident but nine hairs ranged from 60 to 80 μ m in diameter (mean 70 μ m).

In attempting to draw conclusions from this evidence, the tendency is to think of the modern elephant as having lost the hair seen in the mammoth, but the modern elephant must

be regarded as the ancestral form. Thus the ancestral elephant probably lacked skin glands and hair erector muscles, and it seems that these were not developed by the mammoth. Erector muscles, but not the skin glands, would have been advantageous in a cold climate. Why the elephant lost these glands when they are retained in the similarly 'hairless' rhinoceros is not clear.

From the earlier descriptions it seemed that the longest and coarsest hairs of the mammoth could have grown in the central follicles of primary trios, and so be 'bristles', while the shorter and less coarse hairs might have grown in the two laterals, being therefore 'awns'⁶. Although the modern elephant has central and lateral primaries, the hairs differ little in size, and both compare with the longest and coarsest 'bristles' of the mammoth. Evidence of even larger follicles was found in the mammoth, but these did not seem to belong to trio groups. Since at least some of the centrals in the elephant may be tylotrich follicles, the largest hairs of the mammoth could also be tylotrichs. The development of these sensory hairs in the elephant could be associated with the thick and apparently insensitive epidermis, and they could have been retained in the mammoth. The mammoth acquired a greater 'primary' follicle density not only by reducing the distance between the central and lateral follicles within trios, but by reducing the distance between the groups. The resulting greater hair density was associated with a reduction in the hair diameter of the 'primaries' (Table 2), which is a common phenomenon in sheep⁸.

The modern elephant lacks an undercoat and so has none of the smaller secondary follicles that are developed later than the primaries. Such an adaptation to a hot environment is seen in the domestic 'hair' sheep (for example, the southern Desert type of India) which has less underwool as a result of a reduction in the number of secondary follicles². The mammoth redeveloped an undercoat by the acquisition of smaller follicles lacking in the elephant (Fig. 1).

It is tempting to regard these smaller follicles as true 'secondaries', having developed later in the foetus, but unfortunately the lack of glands removes one distinguishing

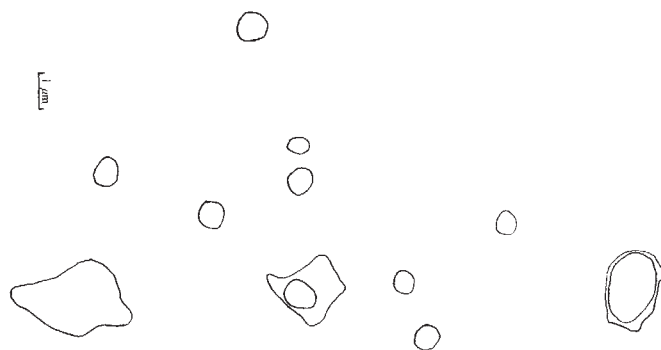


FIG. 1 Tracing of hair group in skin from leg of Liakhov mammoth.

feature⁶. The undercoat usually forms a distinct group of hairs in the diameter distribution which are separated by a wide gap from the much coarser outer coat hairs, as, for example, in the wild sheep². In the mammoth (Table 2) it is difficult to detect any gap, so that the diameters seem to have a continuous distribution.

It may be therefore that once secondaries have disappeared in evolution, they cannot be redeveloped, and so any new undercoat must be formed by increasing the number of follicles, and extending the diameter range, as seems to have happened in the mammoth. An objection to this interpretation is that the size difference between the primaries and secondaries is thought to be due to their development at different times and to follicle competition⁶. If the smaller follicles of the mammoth were developed later, they could therefore be regarded as secondaries.

I thank Dr M. R. Brambell of London Zoo and Mr R. E. Chaplin of Edinburgh Zoo for supplying samples of modern elephant hair, Dr S. K. Eltringham of the Uganda Institute of Ecology for some elephant skin, Dr L. Ginsburg of the Palaeontological Museum, Paris for mammoth skin, H. B. Scott and F. M. Hay for technical assistance, and Dr G. C. Priestley for comments on the manuscript.

Note added in proof: After this letter had gone to press two more small samples were received from the USSR. Seven hairs from the Levar mammoth ranged from 14 to 39 cm in length, and from 98 to 260 μm in diameter with a mean of 179 μm . Three hairs from the Bereleh specimen ranged from 35 to 50 cm in length, and from 240 to 380 μm in diameter. The underwool of this sample ranged from 20 to 46 μm with one hair 70 μm in diameter, and an overall mean of outer hairs and underwool of 57 μm . These are evidence of the longer and coarser hairs mentioned in previous reports, but not found in the other samples of the present study.

M. L. RYDER

ARC Animal Breeding Research Organisation,
Kings Buildings,
Edinburgh EH9 3JQ

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appearance of fields of small speckles which seem to dart about at random. When, however, the noise is viewed monocularly, the texture of the field seems finer and the speckles more nearly static. The display seen monocularly has the appearance of a field of smaller speckles which scintillate *in situ*. I have shown the effect to about ten friends and colleagues, without telling them what to expect. All noticed some difference between binocular and monocular viewing and several promptly described exactly what I had seen. In general, the size change was more consistently reported than the motion change.

I have made a rudimentary effort to explore the effect using a noise display generated by passing the green (514 nm) beam of an argon-ion laser through a pair of ground glass plates, one of which could be translated at a controlled speed. The speckle pattern so generated was projected on a screen. The resulting field of wideband, randomly changing visual noise bore a strong resemblance to television 'snow', though its statistics were not identical⁴. Under the conditions of the arrangement the speckle pattern produced by interference at the retina was so fine that it was nearly invisible, and so could not be confused with the intended display.

The following observations were made by three people. (1) The difference between monocular and binocular viewing was clearly evident, and resembled the effect obtained with the television screen; excessively rapid translation of the ground glass, however, did diminish the effect. (2) There was no difference between monocular and binocular viewing appearances of the stationary noise field obtained when both ground glass plates were still. (3) The effect could be obtained when the images in the left and right eyes were uncorrelated. Such images were obtained by presenting a different portion of the display to each eye using cardboard tubes to facilitate binocular fusion. (4) The effect could also be obtained when half the visual field of one eye was blocked by a card. In this case, one half of the field consists of the noise display as seen monocularly, and the other half consists of the same display as seen binocularly.

The last observation indicates that the effect is not induced by any process occurring in the eye itself, such as a transient misfocussing caused by the change from one mode of viewing to the other. The effect is therefore Cyclopean² in nature. Since it can only be seen in a non-static visual noise field, the classification 'kinetic Cyclopean effect' seems appropriate.

That the effect can be obtained whether or not the noise is binocularly correlated might suggest that even when the binocular images of the noise fields discussed here are correlated, they in some way exceed the ability of the visual processor to perceive the correlation. In any case it is not clear why a planar field of randomly changing visual noise should not appear the same to two eyes as to one. An adequate explanation of this effect might yield new information about the functioning of the visual system.

R. I. MACDONALD

Faculty of Engineering,
Carleton University,
Ottawa, Canada K1S 5B6

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Kinetic Cyclopean effect

WHILE watching noise displayed on a television screen recently I noticed a visual effect which I report here. As normally viewed, displays of video noise ('snow') have the

¹ Nagata, K., and Umehara, T., *Japan J. appl. Phys.*, **12**, 694 (1973).

² Julesz, B., *Foundations of Cyclopean Perception*, (University of Chicago Press, Chicago and London, 1971).

book reviews

Express emotion

Darwin and Facial Expression: A Century of Research in Review. Edited by Paul Ekman. Pp. xi+273. (Academic: New York and London, December 1973.) \$17; £8.

In recent years there has been a new spate of popular books dealing with man's animal origins. These, together with claims about racial differences in intelligence and the biological basis of sex differences, have reinstated the old 'nature-nurture' controversy within psychology, and have led to heated disputes which are unlikely to be scientifically fruitful so long as the question is seen in terms of absolutes rather than matters of degree. Ekman's collection of papers is therefore doubly timely. Not only does it assess the century of research which has followed Darwin's valuable and delightful book, *The Expression of the Emotions in Man and Animals*, but it also provides an object lesson in how the confrontation of genetic and environmental viewpoints can lead to advances beyond the usual, and vague, 'interactionist' compromise.

Taken together, the chapters give a balanced picture of Darwin's general approach to the explanation of behaviour, his specific theories of expression, his research methods, and the data he collected. They show that his position was in fact a sophisticated one, although he emphasised the genetic links between animal and human expressions, and the apparently innate universality of many of our emotional responses. In fact he was sensitive to many methodological pitfalls in research on emotion that later investigators have often overlooked. The present volume presents what is, by and large, a vindication of Darwin's insights. It also shows that we are only just starting to make significant advances on what he discovered a century ago.

Ekman's collection concentrates on the face, mentioning other aspects of expressive behaviour and the structure of emotion itself only in passing. Of the four main chapters only that by Petrino-vich takes a broader view, giving an interesting presentation of how Darwin's theoretical and methodological paradigms have influenced psychology and ethology, and emphasising the importance of a functional view of human activities which takes full account of their biological and ecological context.

Chevalier-Skolnikoff reviews research

on primate expressions, discussing their determinants and functions and relating them to the evolution of facial musculature and of the nervous system. Charlesworth and Kreuzer review the development of expressions and their recognition in infancy and childhood, remaining firmly at the descriptive level because they believe there is too little data to support elaborate theorising. It must be said, however, that on the topics of infant social smiling and fear of strangers there are other data which do go some way beyond Darwin's work and surely permit some theorising about underlying processes.

The other major chapter is Ekman's thorough review of cross-cultural studies, although some other aspects of adult expression are scarcely represented in the book at all. Recent studies, especially his own, contradict the long-standing view of anthropologists that Darwin was wrong in claiming an innate basis for emotional responses. In both Western-influenced and isolated cultures 'basic' emotions like happiness, disgust, surprise, anger, sadness and fear are displayed and recognised in relatively universal ways. Ekman's major contribution is a theory which accepts an innate basis to the linkage between eliciting stimuli, felt emotions, and behavioural expressions, and yet can deal with individual and cultural variations by reference to learned 'display rules' and other complicating factors. Although his theory gives a plausible role to both nature and nurture, and is to be welcomed, it is unfortunate that he does not entirely do justice to the environmentalists.

In fact the book as a whole takes too little account of two important facts about expressive behaviour. One is that facial expressions occur in association with other bodily movements, vocalisations, and even speech. Such multi-channel activities are simultaneously organised at several levels of instantaneous and temporal structuring, as the linguistic anthropologists have correctly emphasised. The second, more crucial point, is that these behavioural performances normally occur within, and are perceived within, a context of continuing social interaction. In fact much non-verbal behaviour serves several functions other than the purely expressive one. Darwin was certainly aware of the first of these points, and would surely have been glad to accept the second, so it is a pity that Ekman's book lacks a further chapter

on the empirical and theoretical evidence relating to them. In fact it is on these topics that the most interesting nature-nurture questions now rest.

Despite these strictures, and other minor defects of the volume, Ekman has produced a valuable book which should be widely read. The contributors' careful avoidance of undefined technical terms, coupled with its very general lessons, should extend its appeal beyond the confines of the specialist disciplines represented. It is therefore inexcusable that its absurdly inflated price should threaten to deter many readers who would learn much from it.

IAN VINE

Critical approach

Exercises in Chemical Physics. By J. R. Riter, jun. Pp. xiv+313. (Gordon and Breach: New York and London, 1972.) n.p.

SIGNIFICANTLY new ideas in science teaching are rare and by no means necessarily translatable into book form. Professor Riter's method consists in a kind of inspired exegesis of selected papers in his field, the broad and delightfully ill-defined area of chemical physics. The papers he treats, or perhaps one should say draws upon, all appeared in about the last ten years of the *Journal of Chemical Physics*, a convenience which by no means takes away from the author's breadth of vision. In all some 166 articles are discussed under headings which include: thermodynamics, quantum mechanics, statistical mechanics, rate processes, mass spectroscopy, infrared, optical, rotational and NMR spectroscopy and the states of matter.

I used the term 'inspired' above quite advisedly. Professor Riter's studies are far more than a simple run-down of the background theory followed by routine, examination-type questions. The content of each article and the student's awareness of it are continuously probed as the author puts questions, makes suggestions for further reading and points to cross-connections. The main results of the article are usually presented in facsimile and a short precis of its aims and achievements given; thereafter the student is asked to reflect, criticise, calculate and cross-refer—sometimes even invited to find mistakes in the original.

The result, as will have been deduced from the number of papers given, is

extremely condensed; with almost every other sentence in some passages putting a question or making some pertinent demand on the student, the quantity of food for thought provided is enormous and quite out of proportion to what one would expect from a book of such modest size. That the result is not indigestible owes much to Professor Riter's sense of style and feeling for the subject matter. His questions have a provocative and searching flavour difficult to convey here, which would be altogether more cramped in a conventional textbook. There is a droll humour, an unwavering sense of relevance, an infectious but entirely mature enthusiasm for chemical physics as a field and a sympathy with the authors whose work is dissected in such fascinating detail.

This adds up to one of the most successfully achieved ideas in advanced teaching that I have seen in a long time, and one which could no doubt be copied, though perhaps not so suitably, in many other branches of science.

M. R. HOARE

The nature of sleep

The Functions of Sleep. By Ernest L. Hartmann. Pp. ix+198. (Yale University: New Haven and London, January 1974.) £3.50 cloth; £1.25 paper.

THIS is a short readable book which should often succeed in interesting and informing the non-expert as well as the specialist. The author has been a prominent worker and prolific writer in the field of sleep research for over a decade. The emphasis in much of the book is on his own sleep research findings and he concludes by advancing yet one more theory concerning the nature of sleep. This latter is contained within the now generally accepted proposition that sleep has a restorative and reprogramming function. In contrast to some others, Hartmann's view is that the former is associated with slow wave sleep wherein synthesis of protein, RNA and other macromolecules crucial to cerebral function occurs, while the latter process is related to paradoxical or D sleep which follows on slow wave sleep and within which reconstruction of memory at the synaptic level can then occur.

Hartmann builds up his case systematically in the early chapters. Other theories are given brief and initial attention before a series of chapters within the rump of the book is given over to the various experimental approaches that can be made. Sleep deprivation studies are presented as having proved to be of limited heuristic value. Consideration is given to his own investigations of personality differences between long and short sleepers, and also to the clinical correlates of variable

sleep which include behavioural, affective, nutritional and occupational items. Some physiological and chemical accompaniments of the various sleep stages are described as are also the ways in which their manipulation can affect sleep in animal studies and the human. A brief but interesting chapter on the different kinds of tiredness and a final one in this section concerning the nature of dreaming serve to remind the reader of the author's firm clinical roots.

The final chapter of the book concerns the integration of his theory of sleep into a more extensive one concerning the nature of the mind and he outlines the possible chemical bases of a variety of psychological mechanisms. Although speculative, it also reflects an important attempt to mobilise psychoanalytic concepts in physiochemical terms in a way which opens up the prospect of experimentation.

For all its chauvinism this is an attractive monograph in which the author, from a position of wide experience in the field of sleep research, has come clean to stoutly defend his holistic and multi-faceted approach and the need for it at this stage in our attempt to understand the chemistry of mind.

The style in which the references are set out (placed at the bottom of each page as well as in an alphabetical list at the end of the book) also makes for easy reading.

A. H. CRISP

Text on bacteria

Fundamental Principles of Bacteriology. By A. J. Salle. Pp. x+1094. (McGraw Hill: New York and London, May 1973.) £8.85.

THIS book is intended for "introductory majors courses in microbiology or bacteriology". But although there are chapters on yeasts, moulds, bacterial viruses and viral diseases of man, it cannot be regarded as a satisfactory microbiology text because protozoa and algae are scarcely mentioned in their own right and because, in the chapters on various aspects of the environment, microorganisms other than bacteria are more or less completely ignored. The short chapter on associations of bacteria does not compensate for these shortcomings and the book must therefore be judged as a bacteriology text.

In the preface the author states that he "attempts to include only sound fundamental material" and that he places emphasis "on the use of chemistry for a clearer understanding of the composition of bacteria and the reactions they produce". The extent to which these aims are achieved varies, of course, but in some chapters there are striking differences in the treatment of comparable topics. For example, the composition and

chemistry of the bacterial cell wall are inadequately treated. There is no mention of teichoic acids although the existence of these polymers is acknowledged in the previous edition. Does the author now doubt the soundness of the work on their composition and structure? In contrast, immediately following the section on the cell wall there is a comparatively full and satisfactory treatment of flagella.

Although the book has many illustrations of chemical structures and equations for reactions, often these do not add very much to the student's understanding of the processes in which they take part. Perhaps the most striking instance is in the chapter on enzymes, where there is a 30-page incomplete list of bacterial enzymes "to show how enzymes are named and classified". This is somewhat extravagant if space when many other topics could, with advantage, have been dealt with more fully.

In many instances archaic nomenclature is used. For example, although it is true that some bacteriologists are extremely conservative in the names they use for carbohydrates, surely nobody now uses 'levulose' (*sic*) in preference to 'fructose' these days, and even if they do it must be confusing for any student to find such inconsistent usage as "... sucrose hydrolyzes sucrose to glucose and levulose," (page 354). Another criticism is that some at least of the reading lists are not very well chosen.

The index is inadequate and the layout of the print on the page is irritating in that although there are large outer margins the print approaches the bottom of the page so closely as to appear to be in danger of falling off!

It is remarkable that although the first edition appeared in 1939 most of the one thousand or so pages of this the seventh edition, are still by the original author. Collaborators are responsible for only four of the thirty-one chapters—some eighty-nine pages. It would perhaps have been better had more authors been involved, for clearly it is beyond the capabilities of any one person to be sufficiently up to date in the many different fields that are covered by this book to be able to extract and present the essential information from each area within the time scale required in the preparation of a new edition.

Of course there is a lot of sound, thoroughly well presented material but deficiencies and distortions of relative importance prevent it being a serious contender for selection as a text for an undergraduate course. Perhaps the nicest way to summarise is to adapt the comment made by one British ex-politician about a recently deceased ex-colleague, and say that it is not fundamentally a bad book.

R. C. W. BERKELEY

Red algae

Biology of the Rhodophyta. By P. S. Dixon. Pp. xiii+285. (University reviews in Botany.) (Oliver and Boyd; Edinburgh, October 1973.) £5.

A COMPREHENSIVE review of the Rhodophyta has been wanted for some years now. Professor Dixon has presented an analysis of current knowledge of certain aspects of the biology of these intriguing algae, drawing on information published up to 1972. The clear purpose is to make the reader aware of the deficiencies in knowledge, as well as indicating the advances made.

Within the scope of the seven chapters there are four in particular which present concise and stimulating accounts of key areas in present day research on red algae: morphology, reproduction, life histories and systematics and phylogeny. The chapter on morphology is of especial merit as it illustrates the numerous modifications of the filamentous habit and the potential of further work on morphogenesis both experimentally and with field observations. These approaches enliven a topic all too often avoided by students. The chapters on reproduction and life histories set out clearly the areas of conjecture, fact and theory. Through all of these the author steers a steady course which leaves the reader with little doubt as to what is reliable in the mass of conflicting information. The lines of future research are also clearly shown. Although the deficiencies in some aspects of the classification system are recognised, the lack of credible alternatives at present is also underlined.

Here then is a book of fundamental importance to students (of whatever age) of red algae. Minor blemishes there are. There is some repetition in later chapters of points covered in the introduction. Many of the diagrams are 'old friends' and some new interpretations would have been valuable. Many readers will regret the lack of a chapter on ecology. But the basic value of the book to continuing studies on red algae cannot be overemphasised.

A. D. BONEY

Man and society

The Riddle of Man: An Introduction to Psychology. By Richard S. Lazarus. Pp. xii+628. (Prentice-Hall; Englewood Cliffs, NJ, January 1974.) \$10.95.

THIS is an outstanding, balanced and humane multidisciplinary book which is much more than an introduction to psychology as its modest subtitle might suggest. The 'riddle of man' the book takes as its subject matter concerns man's biological nature and its interaction and interdependence with his social and physical environments.

Man's biological make-up is seen as influencing the social order he creates and in turn social patterns are shown to modify the way biological processes operate. But the author does not conceptualise man as a passive responder to environmental stimuli and he explicitly rejects the view that in order to understand human behaviour one has to examine it solely in terms of rewards and punishments which follow actions (as does Skinner). The author defines psychology as the science of behaviour and mental activity—a deliberate choice of words since he considers it as one of man's remarkable qualities that he not only acts but is an observer and critic of his own actions. Psychology is presented as one of many interrelated disciplines—biology, anthropology, sociology and economics, all of which attempt to contribute to an understanding of man and society. The author draws on all these sciences and borrows their insights but his focus is a psychological analysis of the individual's biological and social development, his internal motivation and overt responses, his mental and emotional states and his reactions to stress and change.

The book is unusual for a textbook in psychology in that it tells a coherent and compelling story; it addresses itself to some of the unsolved issues of current—and perennial—human concern and to this end has been organised around four key issues: the physical and social environments of man; the psychology of aggression; prejudice and intergroup hostility; and adaptation versus alienation. This book, then, is problem-centred and continuously forges imaginative links with the student's own world and experience. But, within this framework, it also introduces the reader to a great many basic psychological and social psychological processes, concepts and theories. It presents competing paradigms and theories and makes explicit their assumptions and consequences and hence challenges the student to come to his own conclusions and to forego a premature and too exclusive theoretical commitment of his own—be this a commitment to behaviourism or to Freud or to anything else. Thus, at all times the student is presented with scientific controversies which are patiently and skilfully explored to give the student a sophisticated grasp of the issues. Thus, for instance, in discussing the failure of individuals to cope successfully with the stress of living and to find inner harmony, both the 'illness model' and the 'social learning model' are examined as a basis for understanding adaptive failure.

This book is beautifully produced, superbly illustrated with imaginatively chosen photographs, cartoons and diagrams. A further feature are quotations

in the margins from important primary sources and detailed notes on theoretical, methodological and empirical issues which provide a useful underpinning of the text.

HEDY BROWN

Semiconductors

Semiconductor Physics. By Karlheinz Seeger. Pp. xv+514. (Springer Study Edition.) (Springer-Verlag; Vienna and New York, 1973.) 430 schillings; 60 DM.

"THIS book has been designed primarily as a text-book for a three-semester, three-hour per week senior or graduate course in semiconductor physics for students in electrical engineering or physics"; to quote its preface. The course is no easy option, for the book covers with encyclopaedic thoroughness nearly every aspect of semiconductor physics, in breadth if not always in depth. After introductory chapters on elementary properties, band structure and semiconductor statistics, the following topics are discussed: charge and energy transport in a non-degenerate electron gas; carrier diffusion processes, with a brief treatment of transistors; scattering processes in a spherical one-valley model; charge transport and scattering in the many-valley model; carrier transport in the warped-sphere model; quantum effects in transport phenomena; impact ionisation and avalanche breakdown; optical absorption and reflection; photoconductivity; semiconductor light generation; surface properties; and miscellaneous semiconductors. The text is well illustrated with 372 figures, many taken from the literature. There are five useful appendices and author, subject and substance indices.

There are two features which are unusual in a book of this nature. Complete derivations, including the intermediate equations, are given in the text of the numerous mathematical relations. This feature, which is designed to assist the less numerate student, unfortunately increases the algebraic content of the book at the expense of its physics content. The second feature is the provision of over 650 references in the text, many of them to books of similar size. In most cases these references provide a useful supplement to information already given in the text. In some cases, however, they are used as a substitute for such information. The important semiconductor technique of zone refining is mentioned on pages 1 and 153. In the first case it is accompanied by a reference to three books and several other volumes, and in the second by a reference to a book in German. In neither case in any explanation given of the nature of zone refining, an omission ac-

ceptable in a research paper but not in a textbook.

Despite these blemishes, the book provides a well written, comprehensive account of the transport and optical properties of semiconductors, which should prove useful not only to 'senior or graduate' students, but also to research scientists in the area of semiconductor physics.

J. B. BIRKS

Leukocytes collecting

Chemotaxis and Inflammation. By Peter C. Wilkinson. Pp. viii+214. (Churchill Livingstone: Edinburgh and London, 1974.) £4.

THERE is an analogy between the way in which research on leukocyte chemotaxis has been conducted and the movement of leukocytes themselves. For years there was more or less random observation by pathologists, bacteriologists and immunologists, who reported chemotactic activity for neutrophils or mononuclear phagocytes in a variety of products of bacterial growth, tissue injury and immune reactions. A distinction was made between cytotoxins, which themselves stimulate the directional movement of cells, and cytotoxic agents, which bring about a chemical change in non-chemotactic molecules, usually serum constituents, as a result of which cytotoxins are generated. From all this work a long list of chemotactic factors has accumulated, some of which are controversial.

Two technical innovations improved the quality of research in this field. One was the introduction of the Boyden chamber and developments of it which made observations on chemotaxis more quantitative. The second was the availability of new methods for purifying chemotactic factors such as complement components. At present research on chemotaxis is gaining fresh impetus. Random activity and accumulation of miscellaneous observations is now being replaced by studies directed towards the purification and characterisation of cytotoxins and ascertaining how they act on leukocytes. Although the progress so far made is limited, the direction is obviously right.

Some cytotoxins, such as α_s -casein and the cleavage products of complement components, C3a and C5a, have been identified and quite a lot is known about the chemical composition of casein and C3a. The first steps have been taken towards defining the physico-chemical properties of a molecule that make it cytotoxic. Wilkinson's own research on the generation of chemotactic activity when proteins are denatured or coupled with acid anhydrides or other groups is a contribution in this direction.

It seems as though basic peptides

with specific conformations, such as the arginine-rich C3a, or proteins with rather high concentrations of surface non-polar groups, such as α_s -casein and denatured or substituted proteins, are cytotoxic. Possibly the former interact with and cluster certain acidic glycoproteins in the surface of the leukocyte whereas the latter penetrate into the membrane of the leukocyte. Both could increase the permeability of ions, such as calcium, through the leukocyte membrane, thereby triggering chemotactic responses.

Books on chemotaxis are out of date and out of print. This year will see the publication of two books on chemotaxis, a multi-author volume edited by Sorkin and the shorter monograph by Wilkinson under review. The latter is a well written introduction to the subject which will provide enough information for all but specialists. Scotland is neutral ground in the skirmishes that have sometimes developed between Switzerland and the eastern seaboard of the United States, and Wilkinson provides a balanced appraisal of the situation. Where available information is confusing, such as in the role of cyclic nucleotides in the responses of leukocytes to chemotactic factors, this is plainly stated. Perhaps observations on activation of guanylate cyclase, too recent to be included by Wilkinson, will help to resolve the problem.

In any case, there is current interest in the motility of cells, which depends on an actomyosin system similar to that in smooth muscle, on mechanisms by which attachment of various substances to plasma membranes can trigger cellular responses and on the behaviour of embryonic and other cells in concentration gradients. These developments in cell biology should soon be applicable to chemotaxis. Wilkinson's book provides a useful summary of the first fifty years of research on chemotaxis and can also serve as an introduction to the next phase of development. Perhaps it will stimulate cell biologists to use leukocyte chemotaxis as a model system which is of interest in itself and also relevant to medicine.

A. C. ALLISON

Climatic change

Éléments de Paléoclimatologie. By Raymond Furon. Pp. 216. (Sciences de la Terre.) (Librairie Vuibert: Paris, 1972.) n.p.

THERE is a clear need for a new objective, systematic review of palaeoclimatology, but Furon's contribution, instead of improving on the three main books is more likely to retard this interdisciplinary science. It commences with a chapter of statements about the characteristics of present climatic zonation, but without emphasising the underlying

causes, and is followed by an inadequate outline of uncomprehended theories that could cause climatic change. Forty-nine pages are given, according to the title, to methods of palaeoclimatology. They turn out to be seriously incomplete yet space is wasted returning to arguments about continental drift instead of making necessary qualifications to bald statements such as striated and polished stones proving a glacial origin; illite, mica and feldspars in shales associated with Carboniferous coal being incompatible with a humid, tropical climate; and so forth. The objective evaluation of climatic indicators should be possible irrespective of the status of theories of the Earth's surface evolution.

The second section comprises five chapters dealing with different time spans which contain patchy lists of stratigraphic features, only some of which are of palaeoclimatic usefulness—for example, the thickness of Mesozoic volcanics in Mozambique is surely less climatically significant than the coral reefs of the North American and European Permian that are neither mentioned nor shown on a map purporting to show their distribution. Despite his own warnings, the author interprets all occurrences of *Gangamopteris* and *Glossopteris* as indicating warm, humid conditions, and the interbedded glacial debris as always from mountain glaciers associated with an unexplained 300 million year climatic cycle of ice ages. He admits, however, to being "un peu troublé" by the absence of glacial evidence from continents north of the Tethys and sprinkles the section dealing with the Carbo-Permian of Europe with "climat chaude", "chaude et humide", and so on, sometimes based on the presence of evaporites, but often without specifying the evidence. Luckily, the data are plotted on maps using the present geography and so avoid arguments about reconstructions, which Furon disbelieves, and which even those on the plate-tectonic bandwagon must still regard as subject to review.

The 16 palaeoclimatic maps are regrettably small, less than 12 × 8 cm, so that symbols (often only one or two per map) often overlap areas far distant from the actual outcrop and are all the same size, irrespective of such factors as the thickness of the evaporites represented. There is no index, but quite long bibliographies follow each chapter. This arrangement leads to repetition, so that Furon's *La Paléogéographie* is listed five times but Termier and Termier's *Atlas de Paléogéographie* only appears once and Harland's work on the late Precambrian is not mentioned anywhere. Nonetheless, the bibliographies are the most useful part of this unsatisfactory book.

D. H. TARLING

matters arising

Solar activity and climatic change

SIR,—Following John Gribbin's article (*Nature*, 246, 453; 1973), we wish to draw your attention to an unpublished thesis by Dr J. Laszewski (University of Warsaw) which supports Gribbin's suggestions (see also ref. 1). A translation of this thesis is available in the library of The Institution of Civil Engineers.

Laszewski (now professor at the University del Zulia in Maracaibo, Venezuela) has collected long term records of the mean annual discharges of many rivers in Europe, North and South America, Africa and Australia. He demonstrated that the mean annual discharge varies in a cyclic manner which is connected in a complex way with the cycles of sunspots. This is a result of the changes in the atmospheric circulation influenced by the sunspot cycle. The long cycles are divided into shorter cycles, so the story is very complex.

Although none of the records of river discharges examined cover more than about 150 yr and most cover less than 100 yr, one may suspect that the cycle of 200 yr suggested in Gribbin's article is detectable.

Yours faithfully,
T. M. PRUS-CHACINSKI

C. H. Dobbie and Partners,
Francis House,
Francis Street,
London SW1

J. R. D. FRANCIS
Imperial College,
Prince Consort Rd,
London SW7

¹ Stachy, J., *Long term variation of the runoff of Polish rivers* (Institute of Meteorology and Hydrology, Warsaw, 1970) (in Polish).

Refining geoid heights

SIR,—In their recent paper refining the longitude-averaged geoid height with improved values for the zonal harmonics in the geopotential, King-Hele and Cook¹ show their latest version of the height of the meridional geoid section relative to a spheroid of flattening 1/298.25. In this definition an axially symmetric Earth is assumed.

If the distribution of Precambrian exposed and overlaid rocks² is expressed as the ratio of their occurrence along a particular line of latitude to

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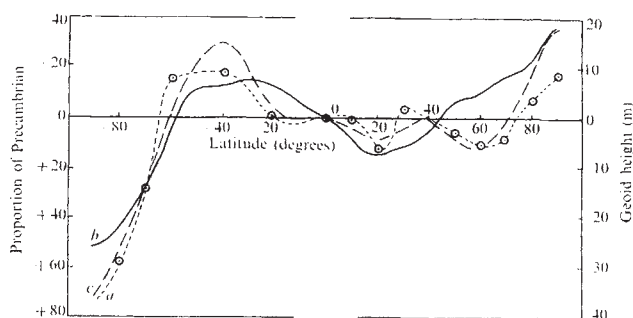


FIG. 1 Comparison of geoid heights given by King-Hele and Cook¹ with proportion of Precambrian stable areas. *a*, Proportion of Precambrian rock; *b*, geoid height, after King-Hele and Cook¹; *c*, precambrian proportion smoothed on double scale.

the total length of that line (that is longitude 0° to 360°) and plotted against latitude, there seems to be reasonable correlation with geoid height. The actual proportions are plotted in Fig. 1 together with a smoothed curve on twice the scale.

A similar correlation exists with longitude for the gravimetric geoid published by Marsh³ which agrees well with the Smithsonian Standard Earth 1969 (ref.

¹ King-Hele, D. G., and Cook, G. E., *Nature*, 246, 86-8 (1973).

² *The Times Atlas of the World* (Times Newspapers Ltd., London 1968).

³ Marsh, J. G., *Significant Accomplishments in Sciences NASA Goddard SFC*, SP-331, 212 (1972).

⁴ Gaposchkin, E. M., and Lambeck, K., *Smithson. astrophys. Obs. Spec. Rep.*, 315, (1969).

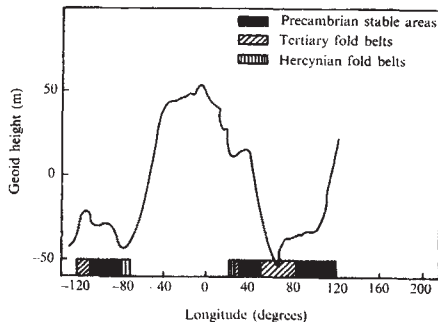


FIG. 2 Detailed gravimetric geoid at latitude 35° N showing correlation with geological formation.

4). The geoid height at latitude 35° N is plotted against longitude in Fig. 2 and the distribution of Precambrian rocks is shown together with the extent of regions of Tertiary and Hercynian folding, which appear to have some significance, particularly in regions of orogeny such as 35° N, 65° E.

The surprising feature of these effects is that the geoid height seems to be lower than the 1/298.25 spheroid in the stable land areas and higher than it in the oceanic areas. This is the reverse of what would have been expected intuitively.

Yours faithfully,

B. P. DAY

Chedworth,
Dippenhall Street,
Crondall, Farnham, Surrey

Left and right in Goya's portraits

McMANUS and Humphrey^{1,2} have described biases in the frequency with which more of the right or left sides of faces are shown in paintings. Examination of 1776 portraits by different artists revealed that, overall, 57% showed more of the left side of the sitter's face. Whilst self-portraits tended to show more of the right side of the face (61% of those examined), male portraits tended to show more of the left side of the face (56%) and female portraits showed an even stronger bias towards the left (68%).

In an analysis of 335 portraits by Rembrandt, the same authors found that the frequency with which more of the left face is shown increases progressively across the following categories of sitter: self, male kin, male non-kin, female kin, female non-kin. To explain this it was suggested that the categories represent positions on a dimension 'socially like myself—socially unlike myself' along which Rembrandt placed his sitters. The choice of mainly left or right face was an indication by Rembrandt as to how he perceived his social relationship with the subjects of his portraits.

Table 1 summarises an analysis of 295 portraits by Goya taken from a comprehensive illustrated account of his works³. Each portrait was classified simply as

TABLE 1 Relative frequencies with which more of the left face is shown in Goya's portraits.

	Self	Male kin	Female kin	Male non-kin	Female non-kin
No. of portraits examined	14	14	12	185	70
No. showing more of the left face	7	9	4	95	50
%	50.0	64.3	33.3	51.4	71.4

showing either full face, or more of the left or right side. The classification of each sitter's relationship to Goya follows that adopted by McManus and Humphrey. The male kin and female kin categories include members of the Bayeu y Subias and Goicoechea families to whom Goya became related through his marriage and that of his son Javier.

Analysis of the data in Table 1 shows, firstly, that Goya's portraits fit the general pattern discovered by McManus and Humphrey in that a majority (56% overall) show more of the sitter's left face. This result is statistically significant (Binomial Test, $Z = 1.98$, $P = 0.024$, one-tailed). There is also a significant sex difference in that, overall, female portraits are more likely to show the left side of the face ($\chi^2 = 4.4$, $P < 0.05$); this result agrees with the original finding by McManus and Humphrey. Analysis of the data in terms of kin compared with non-kin, however, does not reveal any significant relationship ($\chi^2 < 1.0$). More detailed examination of Goya's portraits makes it seem even more unlikely that perceived social relationships influenced Goya's choice of which side of faces to paint: thus of 15 of Goya's self portraits, one is full face, seven show more left face and seven more right; of the four portraits of Goya's son, two show more left face, two more right; all three portraits of Goya's beloved grandson, Mariano, show more left face.

Whilst Goya's works provide evidence which agrees with McManus and Humphrey's interesting general findings about left and right in portraits, their hypothesis relating choice of side and the relationship between artist and sitter is not supported. This could mean either that the effect occurs only with certain artists, or that certain relationships discovered in Rembrandt's portraits are due to other causes, some of which have already been suggested⁴.

Yours faithfully,

I. E. GORDON

Department of Psychology,
The University, Exeter

¹ McManus, C., and Humphrey, N., *Nature*, **243**, 271-273 (1973).

² Humphrey, N., and McManus, C., *New Scientist*, **59**, 437-439 (1973).

³ Gassier, P., and Wilson, J., *Goya, His Life and His Work* (Thames and Hudson, London, 1971).

⁴ Corlett, T., McGlone, B., and Wheale, N. H., *New Scientist*, **59**, 581-582 (1973).

Announcements

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